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SECTION OF CHEMICAL SCIENCES

SEMICONDUCTORS AND CATALYSIS*

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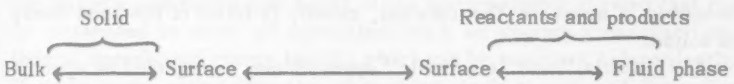
The basic problem in heterogeneous catalysis is the prediction, on the basis of a knowledge of the structure and properties of a solid (the catalyst) of the rates of various types of chemical changes to be observed in a contiguous, generally fluid, phase. No completely satisfactory general solution has been found: the correlation between bulk properties of the catalyst and catalytic activity is often tenuous, ambiguous, or complicated. This paper discusses the reasons for these difficulties and limitations, as well as some steps that can be taken to minimize them.

The problem can be represented schematically by the following "block-diagram":



More or less direct influences connect adjacent "blocks" only, while the correlation sought is between the first and last one.

Especially troublesome is the relation between the last two "blocks": that between the over-all reaction actually observed and the reactions on (and with) the surface that correlate with the surface properties. It is obvious that even a very simple overall reaction may be composed of a very large number of elementary steps (such as ad- and desorptions, surface diffusions and reactions), whose rates may influence each other and any of which may be rate-determining. Recently infrared techniques[1] have made it possible to identify and study species on the surface directly rather than indirectly; however, it is by no means certain that the major surface species so identified are indeed the most important intermediates in the reaction. To remove this source of ambiguity it is important to study reactions involving a minimum number of chemically different species, such as the para-ortho hydrogen conversion that involves only one chemical entity.

*This paper, illustrated with slides, was presented at a meeting of the Section on March 7, 1961. The research described in this article was supported in part by a grant under contract No. Nonr-839(24) with the Office of Naval Research, Washington, D. C.

The Section of Geological Sciences held a meeting on March 6, 1961, at which Clifford A. Kaye of the United States Geological Survey, Department of Interior, Boston, Mass., presented a paper entitled "Geology and Erosion of Gay Head, Mass." This paper will not be published by the Academy.

Two main, very different, guiding principles have been employed in the attempt to systematize the correlation between the properties and structure of the catalyst and its activity. The first, which may be called "spatial," places main emphasis on the steric configuration of the atoms in the various exposed surfaces of the catalyst and their possible congruence with the configurations of atoms in the reactants and/or products. Balandin's multiplet theory[2] is a refined and elaborated example of such an approach.

The other main approach, which can be designated as "electronic," starts from the fact that all catalytic reactions are accompanied by chemisorption, that is, some highly energetic interaction between catalyst and substrate; this must involve some partial or complete electron transfer between them and therefore depend on their electronic structure. An early example of this approach is Dowden's[3] correlation of catalytic activity with the d-band vacancies in metals and alloys.

Two variants of this approach can be distinguished: the "chemical," localized one, which focuses attention on the "bond" formed between the substrate and individual atoms or atom groups of the catalyst, and the "physical" one that deals chiefly with the energetic structure of the assembly of atoms in the catalyst, usually in terms of the band theory of solids.

The "spatial" and "electronic" approach may be regarded as approximations, starting from different points of view, to a more general theory; each emphasizes a particular aspect with relatively complete exclusion of the other. Actually, they are really not as mutually exclusive as may appear at first sight; there is an interdependence; for example, it is obvious that the (electronic) energy states of a surface will be crucially influenced by its geometry. Progress toward a unification of these approaches has been made.[4]

For purposes of simplification and in order to gain a clearer insight it would, however, be desirable to separate "spatial" from "electronic" effects; this is not easy since, especially in the case of metals and alloys, satisfactory correlation with either factor is obtainable, indicating an ambiguous situation. In view of this, the study of catalysis on semiconductor surfaces is especially useful: here the "electronic" correlation can be studied in its pure form unencumbered by "spatial" factors, since the electronic character of a semiconductor can be varied extensively over a wide range through the addition of impurities in quantities that are so small that they have no effect on the lattice.

The qualitative connection between the electronic structure, the electrical properties, and the catalytic activity of semiconductors had been recognized some time ago and found expression in the theory of charge transfer catalysis, enunciated first by Wagner and Hauffe[5] and developed further by Garner *et al.*,[6] V'olkenshtein,[7] and others. A con-

venient summary of much material can be found in Hauffe,[8] Schwab[9] and Law.[10] An essential difficulty in the application of the theory to most reactions, stemming from the qualitative nature of the correlation, is a large amount of ambiguity. Thus, for instance, the increase in the reaction rate of $CO + \frac{1}{2} O_2 \rightarrow CO_2$ observed on NiO with increasing p-character fits nicely into the picture if it is assumed that adsorption on CO, an electron donor, is the rate-determining step, while the *decreasing* rate with decreasing n-character (observed in Zn O) fits equally well if the rate-determining step is adsorption of O_2, an electron acceptor. This again points up the importance of studying simple reactions, with a minimum number of chemical species, as well as of a quantitative treatment that would make possible the expression of the reaction rates of the elementary steps involved in terms of parameters obtainable from theory or independent experiments. A significant advance in this direction has been made by Garrett,[11] with the use of the Shockley-Read[12] model for semiconductor "trapping."

Garrett considers the adsorbed reactants as creating new (localized) electronic levels capable of existing in different charge states ("traps"). In the course of the reaction, charges flow between each of the traps and the valence and conduction bands of the semiconductor at rates that can be expressed in terms of quantities (such as capture cross sections, thermal speeds, and energy levels), which can be measured independently. If these rates, rather than the other steps, such as, ad- and desorption of uncharged reactants and products, surface diffusion, reestablishment of charge equilibrium within the semiconductor, are the slowest, then a clear correlation between electronic properties and reaction rate will exist; if any of the other rates are slow enough to be rate-determining, no such correlation will obtain. One of the most striking results of Garrett's analysis is the demonstration how comparatively limited the range of reaction variables may be within which an influence of the electronic structure of the catalyst might be expected.

Considerable simplification can be achieved if the case of only one kind of adsorbent (trap) is considered. The reaction studied is, then, the rate of ad- or desorption at equilibrium that can be observed experimentally in the case of the para-ortho hydrogen conversion,[13] to which I have already referred. There the surface diffusion and charge equilibrium reestablishment rates can be omitted from consideration (E. M. Loebl, in preparation).

The range of applicability of the correlation between electronic and catalytic properties may be further restricted by the following factors.

(1) Concentration of intrinsic surface states. If these are very numerous they may form a separate (two-dimensional) semiconductor and no interaction with, or correlation with the properties of the bulk semiconductor could be expected.

(2) Different behavior of different crystal faces. The usual catalytic measurements on powdered samples constitute essentially unknown averages over the various crystal faces, each of which is, in theory, characterized by its own set of parameters.

(3) Size effects. The Debye length $(\epsilon \epsilon_0 kT/2\pi ne^2)^{1/2}$ where ϵ is the dielectric constant, ϵ_0 the permittivity of free space, e the electronic charge and n the charge carrier concentration, can be regarded as a measure of the depth to which surface influences penetrate the solid and beyond which bulk properties obtain. For semiconductors, this length may be quite large ($\sim$ 1000 Å); hence in the case of finely powdered catalysts it is not at all clear that there exist bulk properties with which valid correlations can be made.

In our laboratory we are studying the para-ortho hydrogen conversion on the surface of variously doped Ge and Si with widely differing bulk properties (E. M. Loebl and I. Sarfati, in preparation). Preliminary results do not indicate clear correlations either with the reaction rates or their activation energies.

We have also investigated the surface of sodium tungsten bronzes, Na_xWO_3 ($0.3 < x < 0.9$) where the carrier concentration is large and varying widely. Here the rate constant for the p - o H_2 conversion varies by a factor of about 1000, with essentially unchanging activation energy and pressure dependence.[14] The rate constant is not a monotonic function of carrier concentration but rather increases to a maximum at $x = 0.7$ and then decreases sharply. The adsorption of gases such as NH_3 and BF_3 shows parallel behavior.[15]

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SECTION OF BIOLOGICAL AND MEDICAL SCIENCES

THE INITIAL PHYLOGENETIC APPEARANCE OF BONE: AN EXPERIMENTAL HYPOTHESIS*

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Bone is found only in vertebrates. The present fossil record indicates that bone first arose in Ordovician Agnatha. In these fossil fish are found a continuous spectrum of calcified tissues: bone, dentin, cartilage and a type of enamel; these tend to blend insensibly into each other (Ørvig, 1954).

The biphasic processes of calcification of all such tissues in recent vertebrates are essentially homologous. First, a specifically differentiated connective tissue cell (such as osteoblast, and odontoblast) synthesizes the precursors of the fibrous collagen as well as of the mucopolysaccharides of the embedding ground substance, which then undergo extracellular polymerization. It is believed that a firmly bound collagen-mucopolysaccharide complex (a glycoprotein) is formed. Calcification of this organic matrix occurs subsequently. It is currently hypothesized, on the basis of *in vitro* experiments, that the collagen of calcifiable tissues possesses a specific three-dimensional molecular array and consequent electrostatic relations that enable it to initiate the nucleation of the hydroxyapatite salts (Glimcher, 1960). This theory of epitaxy does not, in itself, require the coparticipation of the surrounding ground substance nor, indeed, of the vital cell. Current data, however, do not allow us to rule out the activity of such factors *in vivo*. Nevertheless there now seems little reason to doubt the functional interaction between a specific type of fibrillar protein matrix (or glycoprotein) and a specific type of calcium phosphate salt crystallization in the formation of all vertebrate calcified tissues.

That these processes are presumably identical with those in Agnatha is indicated by the following data. Recent paper chromatography studies of a specimen of Devonian gnathostome fish (*Dinichthys*) demonstrated a

*This paper, illustrated with slides, was the second of three papers presented at a meeting of the section on March 13, 1961. The research described in this article was supported in part by Grant-D-1206 from the National Institute of Dental Research, Public Health Service, Bethesda, Md.

The article entitled "Morphologic and Biochemical Studies of Invertebrate Cartilage Tissue" by Phillip Person of the Veterans Administration Hospital, Brooklyn, N.Y., was the first of three papers presented at a meeting of the section on March 13, 1961. This paper is being considered for publication in the *Annals* of the Academy.

The Division of Mathematics held a meeting on March 8, 1961, at which B. Dunham of the International Business Machines Corporation, New York, N. Y., presented a paper entitled "Symbolic Logic and Computing Machines." This paper will not be published by the Academy.

The Division of Engineering held a meeting on March 8, 1961, at which Jacob Feld, Consulting Engineer, New York, N. Y., presented a paper entitled "Radio Telescope Structures." This paper will be published as an Annal of the Academy.

residual organic residue that was, in part, a collagen degradation product. It had an amino acid content of 3.0 μmole gm. and its principle constitutents were glycine, analine, glutamic acid, leucine, valine and aspartic acid (Abelson, 1957). Our studies of the lower Devonian, *Psammosteus taylori*, have *for the first time* histologically demonstrated the organic bone matrix of Agnatha. The specimen of dermal shield was decalcified with Di-sodium ethylenediamine-tetraacetic acid (0.5 *M* solution) in three changes of 200 ml. each at 4 ° C. embedded in paraffin, and stained with eosin (FIGURE 1). X-ray diffraction studies of a

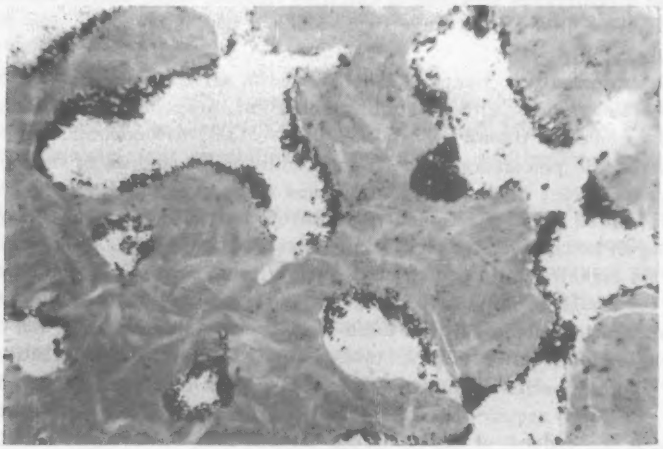

FIGURE 1. The residual organic matrix of a decalcified specimen of the middle layer of the dermal shield of *Psammosteus taylori*. See the text for methods of preparation. The dark granules are residual bits of the embedding quartz matrix. No explanation is offered for the light staining "streaks" within the osseous matrix.

portion of this same specimen, by Aaron S. Posner, National Bureau of Standards, Washington, D. C., has established the presence of a fluorapatite, most probably a substitution of the original hydroxyapatite (FIGURE 2). The presumptive identity of both organic and inorganic bone constituents in fossil Agnatha and recent vertebrates seems to establish a parameter for the processes of skeletal evolution, and permits the following speculations.

The precise nature of the original *external* fluid environment of the first ossified vertebrates (that is marine, brackish, or fresh water) is still a matter of debate. However we may describe their probable *internal* fluid environment with some degree of accuracy.

If bone in Agnatha is homologous to recent vertebrate bone in general, and to fish bone specifically, then the composition of serum in Agnatha

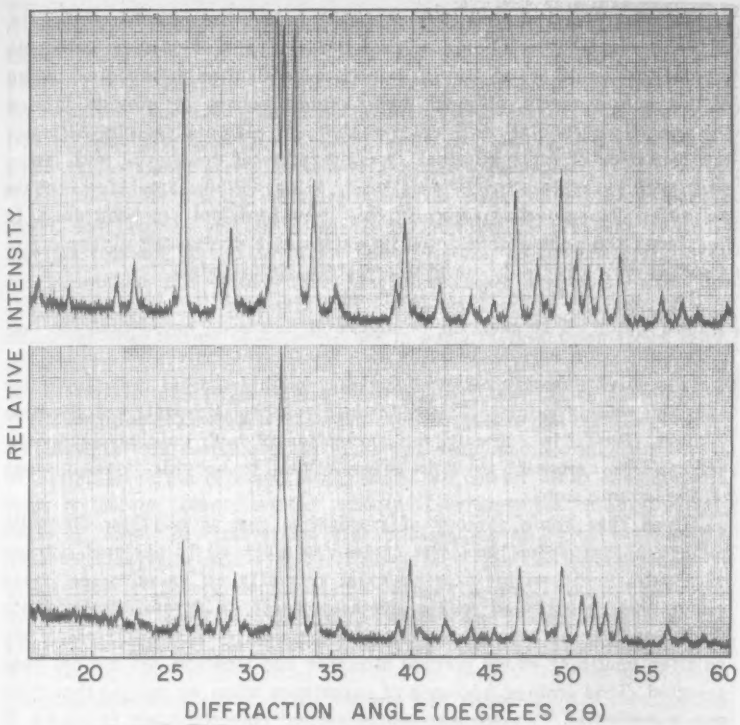

FIGURE 2. Comparison of the powder x-ray diffraction pattern of synthetic hydroxyapatite (upper) and the lower compact layer of *Psammosteus taylori* bone. Patterns taken on a commercial proportional-counter diffractometer using copper K-∝radiation. The bone material is definitely an apatite phase with its *c* axis equal to the hydroxyapatite *c* axis and its *a* axes smaller than the hydrosyapatite *a* axes. The change in *a* axis size could be due to F^- and/or CL^- substitution for OH^-. Please note that the X-ray diffraction peak in the upper pattern at 31.5° 2θ goes off the scale 1½ times the top of the relative intensity scale. Prepared by A. S. Posner, National Bureau of Standards, Washington, D.C.

was probably similar to that of living fish. Current data indicate that the concentration of calcium and phosphate ions, for example, is of the same order of magnitude is elasmobranchs, teleosts, and mammals (Bailey, 1957; Vinogradov, 1953). When we understand the metabolism and homeostasis of minerals in fish more fully we may probably infer homologous processes to the Agnatha.

Calcification in invertebrates appears to be basically similar to that described above. Again biphasic processes are involved in the ultimate formation of the characteristic calcium carbonates. In Molluscs the scleroprotein conchiolin matrix of the shell seems to play a role similar

to that of collagen in bone (Wilbur, 1960). Indeed the recent experiments of Watabe and Wilbur (1960) indicate that the type of calcium carbonate crystal (aragonite or calcite) is, in part, dependent on the specific nature of the protein matrix, although these authors cannot yet provide data on the specific molecular configurations that may play such a role. Although the formation of glycoproteins, by conjugation of conchiolin with mucopolysaccharides is strongly suggested, Wilbur (1960) concludes that the definitive experiments to support this view have not yet been done. In Crustacea the polysaccharide chitin exists as a conjugated glycoprotein, a part of which is firmly bound to a protein, arthropodin.

Such glycoproteins are at least generically similar to those of vertebrate calcified tissues. In crustacea it has been noted that the calcium salts enter into combination with the organic molecules and further, that "a polysaccharide sulfate complex, possibly critical in configuration with the protein moiety..." may initiate calcium carbonate calcification (Travis, 1960). In summary, the nucleation of both calcium phosphate and calcium carbonate crystals seems related to specific configurations of the organic matrix.

Given this basic analogy of mechanism, let us consider the salts produced. Mammalian bone may contain as much as 15 per cent calcium carbonate, believed by most to exist primarily as an adsorbed phase rather than substituted within the hydroxyapatite crystal. Invertebrate skeletons may contain as much as 15 per cent of a calcium phosphate salt (an apatite?) whose precise structure and location has not yet been reported. This problem becomes of importance when we realize that there are a series of "phosphorus-concentrating" invertebrates (FIGURE 3) in which the CO_3/PO_4 ratio reaches 0.5 and less (Vinogradov, 1953). The possibility exists that in these forms there are two types of organic fibers (or glycoproteins), each specifically engaged in the nucleation of a specific calcium salt. This is very intriguing and highly suggestive. It should be noted that while vertebrate and invertebrate collagens differ in several significant ways (Gross and Piez, 1960), there is "a structural

PORIFERA	*Leptogorgia, Phyllogorgia*
COELENTERATA	CERAMOPORIDAE, *Favositella*
BRYOZOA	*Bugula, Bolopora*
BRACHIOPODA	*Lingula, Obolus* and others
VERMES	*Hyalinacia, Onuphis, Leodice*
MOLLUSCA	CONULARIIDAE, HYALITHIDAE
ARTHROPODA	DECAPODA, STOMATOPODA, MALACOSTRACA, AND OTHERS
VERTEBRATA	ALL

FIGURE 3. A list of phosphorus concentrators whose skeletal parts contain phosphorus. Taken from Vinogradov (1953), take 258.

relationship" between the mesodermal tissues of forms ranging "from higher vertebrates to coelenterates and sponges" (Marks, Baer and Blake, 1949). Should our proposed studies establish a "typical" collagen-apatite relationship in these invertebrates we should be in a position to speculate more meaningfully about the factors that lead to the initial phylogenetic appearance of bone in the vertebrates.

Exactly what did the vertebrates do, *in terms of calcified tissues alone*, that was so unique? It seems that neither the presence of cartilagelike tissues, in the broadest sense (Person, 1960; Schaeffer, 1930) nor of apatite salt calcification, nor of collagen or glycoproteins are uniquely vertebrate characteristics. Even the gross and microscopic structural arrangement of exoskeletons in some invertebrates are at least superficially analogous to some vertebrate calcified tissues (without any attempt to infer phylogenetic relationships), that is, the exoskeleton of the decapod crustacea it has been claimed, resembles enamel (Travis, 1960), while the carpace of Limulus has been superficially likened to an Agnathic shield (Patton, 1912). Thus we cannot claim that there is even a unique characteristic structural arrangement of exoskeletal calcified tissue in vertebrates. What does appear to be unique was the production of only one specific type of collagen (or glycoprotein) by specifically differentiated connective tissue cells. This collagen was then able to interact with available calcium and phosphate ions in the serum to initiate the nucleation of *only* an apatite salt. If this is true, it is theoretically attractive to consider the possibility that some phosphate-accumulating invertebrate already has possession of this mechanism together with its carbonate-forming processes, may lie in the line of vertebrate descent (FIGURE 4). It must be distinctly understood that

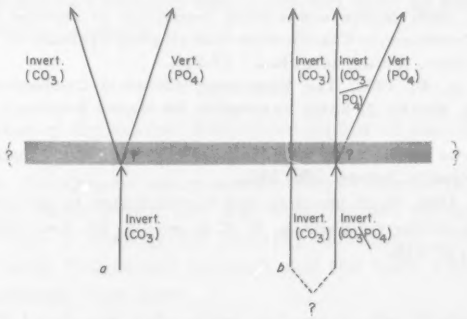

FIGURE 4. Two concepts of the origin of calcium phosphate mineralizing vertebrates. In *a* they derive from carbonate forming invertebrates, while in *b* they derive from phosphate concentrating invertebrates; that is, from forms in which both carbonate and phosphate were laid down. The dark band indicates the era in which this change occurred. It is suggested that *b* furnishes a hypothesis that easily permits further investigation.

phosphate-concentrating invertebrates may well have arisen independently as so many individual evolutionary experiments in mineralization, and that these forms probably do not bear any lineal relationship to each other. Nevertheless, the possibility does exist that one such example of invertebrate biochemical evolution may well have proved successful in the sense that it predicated phosphate-salt formation in the lineally succeeding vertebrates. At any rate this hypothesis permits a series of interesting laboratory investigations.

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DIFFERENTIAL OSSIFICATION IN THE FISHES*

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Cartilage is regarded as an embryonic adaptation chiefly because it is a plastic skeletal tissue that can grow rapidly by internal expansion and thus provide a framework for the developing embryo. It also has been retained as an important component of the skeleton in the adult stage of many kinds of fishes and amphibians. This retention is undoubtedly neotenic as Romer (1942) and De Beer (1958) have pointed out. Is it also reasonable to conclude that the presence of cartilage in the adult with or without bone involves an ancient neotenic adaptation associated primarily with an aquatic habitus? A clue to the meaning of the cartilage-bone relationship may perhaps be found by reviewing briefly the distribution of these tissues in the major groups of fishes.

Agnatha

In the most primitive known vertebrates, called ostracoderms (Ordovician to Devonian), the endoskeleton was generally composed of cartilage (Stensiö, 1927 and 1932), apparently uncalcified.** Bone (covered with dentine and enamel as in most other early fishes) was restricted to the cranial exoskeleton and body scales, except in the cephalaspids, which had a thin layer of perichondral bone surrounding the chondrocranium. The exoskeleton was best developed in the benthonic cephalaspids (the endocranial cartilage was replaced in a few genera by cancellous bone), and in the heterostracans, which were also benthonic. It was considerably reduced or thinned in the free-swimming anaspids. The living, free-swimming agnathans (lampreys and hagfishes) have an entirely cartilaginous, uncalcified skeleton.

Placoderms

The skeleton of the several distinctive groups of placoderms, or first jawed vertebrates, differs considerably in the relative amounts of bone and cartilage. The free-swimming acanthodians (Silurian to Permian) had a cartilaginous endoskeleton that was calcified and partly covered with perichondral bone in some genera (Watson, 1937; Ørvig, 1951). The head was covered with thin dermal elements and the body with tiny scales having a thick basal bony layer.

The early benthonic arthrodires or arctolepids (lower and middle Devonian) had an endoskeleton of calcified cartilage covered with peri-

*This paper was the third of three papers presented at a meeting of the Section of Biological and Medical Sciences on March 13, 1961.

**Calcified cartilage is known from the Ordovician, but only as isolated fragments (Ørvig, 1951).

chondral bone, heavily ossified head and shoulder armor, and ossified dermal mouth parts. Among the brachythoracid arthrodires, including free-swimming and benthonic types, some genera had a calcified endoskeleton covered with perichondral bone, while others, including the largest forms, lost the calcification entirely. All had well developed head and shoulder armor and ossified dermal jaw elements. The presumably free-swimming ptyctodonts had a calcified endoskeleton and perichondral bone locally thickened on the primary crushing jaws (Ørvig, 1960). The dermal head, jaw, and shoulder elements were reduced. The benthonic phyllolepids are known only from heavy dermal armor; presumably the endoskeleton was not calcified.

The remaining groups of placoderms were presumably benthonic. The braincase of the macropetalichthyids was perichrondrally and endochondrally ossified (Ørvig, 1951); the head shield and shoulder armor were heavily ossified and the trunk was covered with scales (absent in arthrodires). The rest of the skeleton was probably entirely cartilaginous. The antiarchs had a thick, boxlike anterior dermal armor and ossified dermal mouth elements. The endoskeleton lacked perichondral bone, and calcified cartilage was restricted to the armored pectoral appendages. In the raylike stensiöellids and rhenanids the exoskeleton was reduced to isolated plates, and the dermal shoulder girdle was also reduced (Stensiö, 1959). The endocranium was partly ossified in one rhenanid, but probably only perichondrally in the other genera. Otherwise the skeleton was probably calcified and at least partly perichondrally ossified (Ørvig, 1951).

Cartilage Fishes

The Chondrichthyes (Devonian to Recent) including the sharks, rays, and holocephalians, lack a bony exoskeleton and have an entirely cartilaginous endoskeleton. Bone occurs only as a component of the osteodentine in the dentition and placoid scales. The endoskeleton may be partly or, more frequently, entirely calcified.

Higher Bony Fishes

In the Osteichthyes or higher bony fishes (dipnoans, crossopterygians, and actinopterygians) the endoskeleton is generally well ossified, although phyletic reduction in ossification is evident in many groups.

The neurocranium of the Devonian dipnoans was completely (Säve-Soderberg, 1952), or partly ossified (Westoll, 1949) but thereafter remained cartilaginous. The dipnoan visceral skeleton became mostly cartilaginous after the Devonian, and the dermal skull was progressively reduced. The postcranial skeleton was variably and thinly ossified in the extinct representatives, and is now mostly cartilaginous. Some Devonian dipnoans had ossified centra (Jarvik, 1952), but otherwise the notochord has per-

sisted without surrounding or replacing ossification (except for thin, cylindrical centra in *Protopterus*).

The rhipidistian crossopterygians (Devonian to Permian) had an ossified braincase and visceral skeleton covered completely by dermal bones. The postcranial skeleton was also entirely bony, with ossifications surrounding the notochord. The neurocranium of the related coelacanths became partly cartilaginous after the Devonian; the visceral skeleton has remained mostly ossified. The fin skeleton has been sporadically ossified throughout coelacanth history; the neural and haemal spines only are perichondrally ossified (Schaeffer, 1952).

The vast actinopterygian assemblage may be divided into three evolutionary levels, the chondrostean, holostean, and teleostean. Although the principal line leading to the teleost level involved a well ossified endoskeleton and dermal skull, side lines within each level show reduction in the ossification of the neurocranium and in the number of dermal elements. The notochord persisted into the holostean level, and constant ossification of the centrum is confined to the teleost level. Thinning of the dermal elements and general lightening of the entire skeleton also occurred sporadically at all levels, but is particularly characteristic of the teleost.

Conclusions

These data can be interpreted to show that the distribution and density of cartilage and bone in the fish skeleton are adaptive and are influenced mainly by four interrelated factors: mechanical stress, equilibrium, buoyancy, and protection (the more general problem of calcium metabolism will not be considered).

In all groups above the agnathan level the endoskeleton is usually strengthened by calcification, perichondral ossification or complete ossification. This must, in part, be related to the stresses of feeding and locomotion. The presence of uncalcified cartilage in the skeleton suggests that the areas involved are subjected to only minor stresses (anaspid endoskeleton), or are effected by factors that override the influence of the stress factor (dipnoan braincase).

A well ossified exoskeleton frequently is developed in benthonic forms (cephalaspids, many placoderms, callichthyid catfishes), but it is also present in some free-swimming types (most heterostracans and some placoderms). Protection is only a partial explanation. Dermal armor also may be related to an increase in specific gravity for bottom dwelling (the existence of a swimbladder or lung below the osteichthyan level is problematical). The free-swimming armored forms probably represent for the most part descendants of benthonic types that have somehow compensated for the armor, perhaps by rising from the bottom only to obtain

food. Well ossified, extinct osteichthyans such as the rhipidistians probably had a density close to that of *Amia* or the gar, which have no appreciable buoyancy problem.

Equilibrium depends largely on the distribution of skeletal and muscular tissue around the center of gravity. In forms with a heavy anterior exoskeleton in which the center of gravity is far forward, locomotion is tadpolelike and relatively inefficient unless the exoskeleton is counterbalanced by the trunk. An example of equilibrium adjustment may be represented by the ptyctodonts which had a reduced exoskeleton associated with a slim, tapering trunk.

Denton and Marshall (1958) have demonstrated experimentally that buoyancy in fishes represents a balance between skeletal tissue and muscle (protein) on the one hand and gas and fat, plus dilute body fluids (in sea water), on the other. It follows therefore that the total amount of calcified cartilage and/or bone* in the skeleton plus the composition of the scales (especially in some of the early fishes) must be adjusted, in part, to the buoyancy requirements. The loss or gain of calcification or ossification involves primarily a compromise between the demands of mechanical stress and of buoyancy.

Selection for neutral buoyancy is evident in many groups of osteichthyans, either with or without a swim bladder (Jones and Marshall, 1953). The chondrichthyans, which lack a swim bladder, and numerous osteichthyans, again with or without a swim bladder, have a body density greater than that of water. Negative buoyancy also must have existed in many extinct groups that had a well-developed exoskeleton (most ostracoderms and many placoderms) and perhaps in some forms with a thin or reduced dermal skeleton (anaspids, stensiöellids, and rhenanids).

Since the sharks have existed for more than 350 million years, it may be concluded that the apparent inadaptive effects of negative buoyancy have been more than offset by the adaptive advantages of a calcified skeleton and a well-developed body musculature. This conclusion should apply, in general, to all fishes with negative buoyancy regardless of the composition of the skeleton and the mass of the body musculature.

In summary, the adaptive significance of the distribution and density of cartilage and bone in the adult fish skeleton must be related to the interplay of at least four factors: mechanical stress, equilibrium, buoyancy, and protection. Calcified cartilage and bone represent two different ways of strengthening the skeleton. The ultimate superiority of bone over cartilage as a stress-resisting tissue is evidenced by the nearly universal occurrence of bone in the Osteichthyes, which have attained the greatest feeding and locomotor efficiency among the fishes.

*Bone has an average specific density of 1.93. Cartilage must vary from nearly that of water to almost that of bone depending on the amount of calcification.

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SECTION OF PHYSICAL SCIENCES

THE USE OF ANALOG COMPUTERS IN THE KINETIC ANALYSIS OF COMPARTMENTED SYSTEMS*

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Modern high-speed computers have been and are being used extensively in physics, engineering, and industry to handle a wide variety of problems. Their applications to biological problems have been slower to develop, although the potentialities are great.

There are two major types of computers, digital and analog. The digital computers handle numbers only and are capable of achieving almost any degree of precision that may be required. The analog computers handle continuous physical variables and in general are less precise than the digital computers but, at least for certain classes of problems, they have the advantage of simplicity and rapidity of response. Analog computers are particularly useful in the stage of searching for the way to work a problem, while after the procedure is established and a volume of repetitive calculations are needed, the digital method becomes more convenient. In addition to the differences in the philosophies of their use, analog computers have the virtue of relatively lower cost, although with either type of machine there does not seem to be any upper limit to the price.**

This paper is limited to the application of analog computers to essentially one problem, that of relating the observable behavior of a tracer to the kinetic behavior of substances in compartmented systems. An explanation of the origin of this problem and establishment of the terminology to be used is in order at this point.

By a compartmented system we shall mean the idealization of a biological system as consisting, for mathematical purposes, of a number of interconnected regions of space, within each of which perfect mixing of its constituents is assumed. The compartments do not necessarily conform to anatomical boundaries but may be defined in terms of cellular, physiological, or biochemical concepts.

Homeostatic mechanisms serve to keep the chemical composition of the tissues constant with respect to most constituents. This does not mean, however, that the composition in terms of the atoms and molecules involved is identical at two different moments. Quite the contrary; the now classic work of Schoenheimer[2] and his colleagues has established the concept of the dynamic state of body constituents. The condition

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**A collection of general articles on analog computers is available from the Philbrick Company, Boston, Mass.[1]

characterized by the existence of a constant amount of a substance within a compartment and with equal rates of flow of the substance into and out of the compartment is called a steady state.

Prior to the advent of isotopic tracers, many steady-state situations were regarded as being inaccessible to investigation because the methods available involved disturbing the steady state and creating nonphysiological conditions that could not be guaranteed to represent the normal steady-state conditions.

In the steady-state, the ratio of the rate of flow of a substance through a compartment (ρ) to the amount present at any moment within (S) it is a number called the turnover rate constant (k). The fundamental assumption in tracer theory is that this turnover rate constant is the same for the labeled form as it is for the unlabeled form of the substance. In some circumstances an isotope effect, due to the difference in mass of the labeled form from that of the natural form, does occur, but usually this effect is negligible. Further discussion of the basic definitions and assumptions involved in tracer theory are available in textbooks on isotopic tracers and in numerous articles in the literature.[3-5]

The relationships between the behavior of a system and that of a tracer in the system are easily developed for the simplest systems. In the one-compartment open system, $\rightarrow 1 \rightarrow$, we have, with x representing specific activity, and assuming no inflow of tracer:

$$\frac{dx}{dt} = -kx$$

which leads to the solution: $x = x(o)\ e^{-\lambda t}$ where $x(o)$ is the initial value of x. In this system the exponent is equal to the turnover rate constant:

$$\lambda = k.$$

For the two-compartment closed system, $1 \rightleftarrows 2$, assuming that compartment 1 only is labeled initially, the solutions involve an equilibrium term, $x(\infty)$, and an exponential term:

$$x_1 = x(\infty) + (x(\infty) - x(\infty))\ e^{-\lambda t}$$

$$x_2 = x(\infty)\ (1 - e^{-\lambda t})$$

In both of these equations

$$\lambda = k_1 + k_2, \text{ where } k_1 = \frac{\rho}{S_1} \text{ and } k_2 = \frac{\rho}{S_2}$$

FIGURE 1*a* illustrates the curves obtained in a 2-compartment system when the data are plotted on semilogarithmic coordinates. FIGURES 1*b* and 1*c* illustrate 2 ways of obtaining λ from these curves by graphical analysis, in the first case by using the ordinate differences between each each curve and the equilibrium value of x and, in the second from the ordinate differences between the 2 curves, giving in either case a straight line, the slope of which is measured by the half time $T\frac{1}{2}$ and from which λ is calculated using the relationship $\lambda = \frac{0.69315}{T\frac{1}{2}}$ Although the turnover

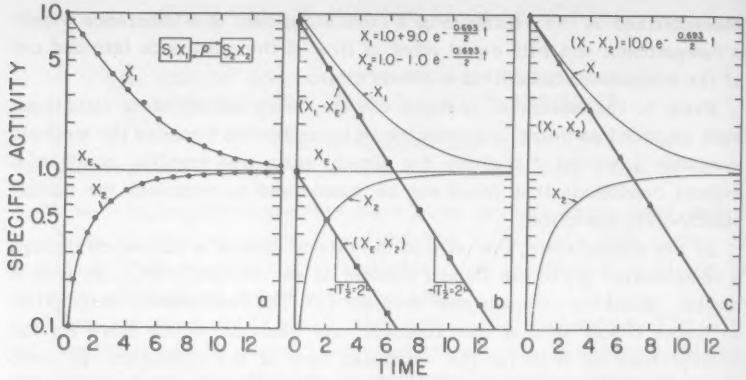

FIGURE 1. Graphical method of determining the exponential component of curve describing data obtained in a two-compartment closed system.

rate constants are now lumped in the exponent λ, it is not difficult to obtain solutions for the flow rate ρ, and for the individual ks in terms of the coefficients and exponents.

In 3-compartment and multicompartment systems, however, the relationships between the λs, which can be determined experimentally, and the ρs, which must be calculated, become increasingly complicated and analysis by desk calculations becomes too cumbersome to be practical.[6,7]

It is at this point that analog computers begin to be helpful. The mathematics of the kinetic behavior of tracers in steady-state systems is, with certain restrictions, formally identical with that of the voltage curves in electrical systems consisting of capacitors connected through resistors. Thus, FIGURE 2 shows the electrical analogs of two typical kinds of biological compartmented systems. This direct type of analog is

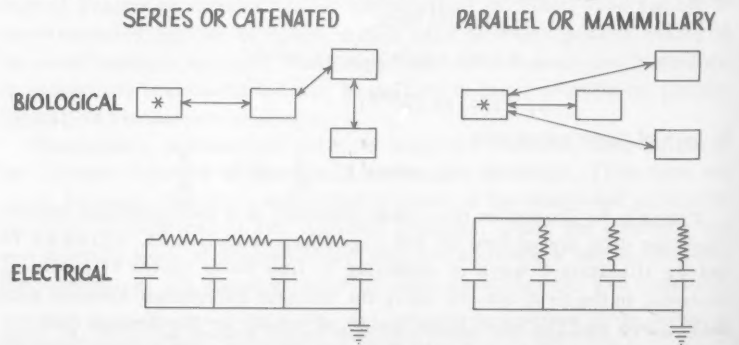

FIGURE 2. Electrical analogs of two basic kinds of steady-state compartmented biological systems. Kinetic analysis of compartmented systems using electrical analog.

distinguished from the general purpose analog computers, which are usually used as differential equation solving devices. Of course the general purpose computer can be used to handle the problems under discussion, but the direct analog is adequate for many problems and is simpler to build and to use.

FIGURE 3 shows the circuit diagram for a 4-compartment analog instrument that was demonstrated in the talk. With this device an initial voltage, representing specific activity of the labeled material, may be applied to any of the four compartments and the simulated specific activity in any of the four compartments may be displayed on an oscilloscope.

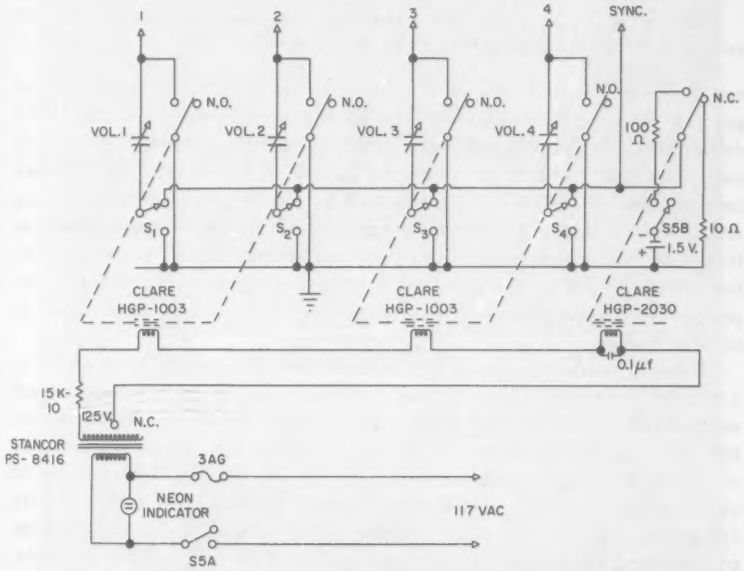

FIGURE 3. Circuit diagram for a simple four-compartment analog. The volumes or sizes of the compartments are represented by 1 uF decade box variable condensors. External 100 K or 10 K variable resistors (not shown) are plugged in to connect the condensors. The circuit provides a repetitive cycle suitable for display on an oscilloscope. Courtesy of R. M. Sugarman.

FIGURE 4 shows some representative curves that actually are based on photographs of the curves generated by a larger analog computer for the types of systems shown but can be obtained with the small analog diagrammed in FIGURE 3.

A more elaborate computer is needed to handle systems having unequal flow rates in the opposing directions between compartments, as occurs in open systems having the route of entry into one compartment

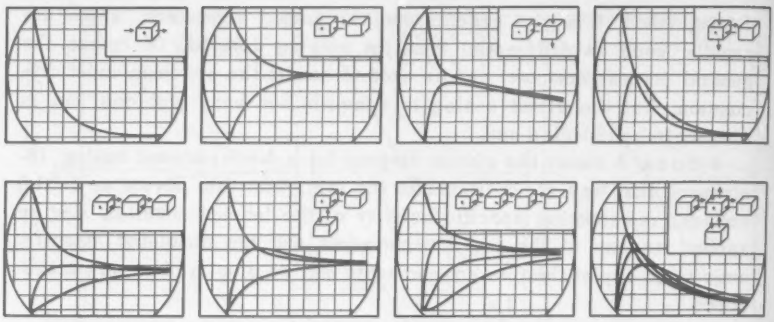

FIGURE 4. Basic types of compartmented systems analyzable with RC analogs and typical curves obtained with an analog computer.

and the route of exit from a different one. It is, however, not necessary to go over to the general-purpose type of analog computer to handle such difficulties. As in the computer described by Macdonald,[8] operational amplifiers may be used in otherwise basically RC circuits to simulate unequal forward and reverse flow rates between two compartments. Among advantages of using direct analog circuits where they are applicable is that it is not necessary to write out the differential equations of the system either to set up the problem or to obtain the answers. This is important when investigators with minimal training in mathematics want to use the device.

A larger analog computer to be described elsewhere[9] is currently in use at Brookhaven. This machine has provision for eight compartments represented by capacitors, which may be connected either through resistors or through operational amplifiers. The voltage curves representing as many as four compartments may be displayed simultaneously on an oscilloscope. In this machine there is also provision for electronically displaying a set of data points, each of which may be set to any location to represent experimentally obtained points. The controls for the data points and those for the analog are independent, so that there is complete freedom in manipulating the parameters of the model system to give a set of analog curves consistent with the data. It must be emphasized that this process is more than an elaborate curve-fitting procedure; in the course of curve-fitting a set of system parameters are being chosen for which there is assumed to be a 1:1 correspondence with the biological system of interest.

FIGURE 5 illustrates an application of the larger computer in the analysis of data obtained in a study of the behavior of labeled creatinine. In this example the initial infusion took 30 min., as shown by the rising portion of the curve. The dots correspond to data points representing the concentration of the labeled creatinine in the plasma, and the analog

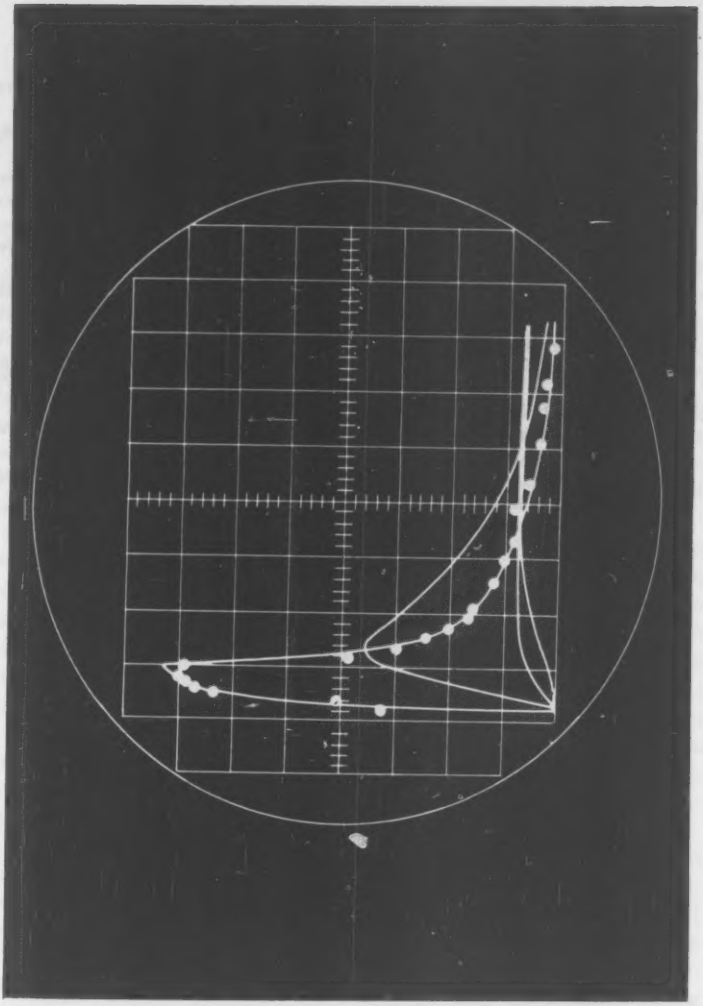

FIGURE 5. Curves fitted with an analog computer to concentration in plasma of creatinine injected intravenously at a constant rate during the first 30 min. Data courtesy of Paul Schloerb.

curve was adjusted to fit these points. The other curves represent radioactivity in other compartments that were needed to give a model system in which the tracer behavior would be consistent with the observed data.

Other applications have arisen in studies of electrolyte and trace metal metabolism, and in studies of the degradation of labeled proteins.

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DIVISION OF PSYCHOLOGY

METHODS, PROCEDURES, AND THE PROBLEM OF CRITERIA IN THE EVALUATION OF COLLEGE GUIDANCE PROGRAMS*

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Review of studies that purport to evaluate college guidance programs reveals, in general, the following approaches:

(1) Surveys of student satisfaction with the services provided by programs have been conducted. These include such studies as client satisfaction with college counseling services and more comprehensive approaches to assess student satisfaction with the entire range of services and functions performed by college personnel staff and the faculty in their advisory relationships. These studies reveal a mixed reaction by the students to the college guidance program, although Iffert [1] reports that college students evaluate such services in their colleges and universities as less useful than the instructional programs in the same institutions.

(2) College guidance programs, including specific services, have been evaluated by comparing their purposes and functions with the needs of students, as these have been identified through the administration of check lists and similar instruments, and with the general purposes of colleges and universities. These studies have provided no really rigorous evaluation of programs, but have been content, in general, to identify a particular service and function that aim at helping the students with one or more major problems revealed in check lists. The student needs that have been surveyed include those with respect to vocational and educational planning and those with respect to personal problems and interpersonal relationships.

(3) The effects of specific techniques have been investigated. Most such studies have attempted to assess the effects of particular counseling techniques to achieve relatively limited purposes. An example is an investigation by Berdie[2] hypothesizing that counseling would (1) reduce anxiety, (2) reduce defensive reactions, and (3) help students solve problems. In a study involving a substantial period of time none of these hypotheses was supported by the Berdie research. Other research on the effects of specific techniques used by counselors has yielded similar inconclusive results.

* This paper was the second of three papers presented at a meeting of the Division on March 20, 1961.

The Division of Psychology held a meeting on March 20, at which Martin Hamburger of New York University, New York, N. Y., presented a paper entitled "The Objectives of Student Guidance." George A. Pierson of Queens College, New York, N. Y. also presented a paper entitled "Results and Achievements to Date: The Failures and Successes of Current Guidance Practices." These papers will not be published by the Academy.

In two reviews of research, one done in 1950 and the other in 1959, Strang[3] notes that research studies of college and school guidance programs are severely limited for the following reasons: (1) there is a lack of specific criteria; (2) there is a lack of scope in the studies; (3) there is a lack of longitudinal approach; and (4) the data tend to be mass data or group data and not studies of individuals.

In an attempt to identify the major characteristics of research in the field and to suggest ways in which it might be more fruitful, Strang concludes, "We need a theoretical framework that takes into account this complex of forces and factors: the individual student's drives and actions, the influence of his behavior on the group, the group's influence on him, and the other forces of the campus and community that help to determine his personal and social development."[4] Thus Strang is noting the lack of a theoretical and conceptual framework as the major limitation to the research done to date.

In two additional reviews of research published in 1960, Lifton notes that "Research in this area has not kept step with the need to assess the effectiveness with which this field is achieving its goal,"[5] and Rothney and Farwell note, "It is evident that the problem of selecting and securing adequate measures of criteria against which guidance services are to be assessed has not been solved."[6]

It seems clear that the criteria characterizing research studies up to this time are inadequate to evaluate college guidance programs. While it is important to gauge student satisfaction with the services and functions of such programs, such studies of them seem to offer only oblique evidence with respect to evaluation of the program itself. On the other hand, the assessment of the effects of specific techniques, although useful, seems inappropriate when viewed in relation to the total diversity of a college student body and in relation to the total guidance program.

A major handicap of the studies to date has been the lack of recognition of the individuality of college students. Thus a criterion that Berdie[7] proposed to measure the effects of counseling, namely the reduction of anxiety, seems to assume a monolithic character of college students with respect to the role of anxiety in their personal lives. Nevertheless, careful examination of the diversity of college students would suggest that while the reduction of anxiety would seem appropriate as a purpose toward which counseling should aim for some students, for others a more appropriate goal of counseling might be the induction of anxiety unless, of course, the college guidance program, including counseling, is conceptualized as serving only a minor part of the student body of any college: in particular, those students who exhibit anxiety reactions that are disabling them. Such a limited view of the purposes of college guidance programs is unacceptable to those who have attempted to describe the purposes of them.

Examination of the literature of the guidance and student personnel field suggests remarkable agreement as to the principal purpose of college guidance work. Simply put, the purpose is to *encourage the optimum development of each individual while recognizing his worth and dignity as a human being.* Cribbin,[8] in a review of more than 200 pieces of literature in the field, noted almost complete agreement on this purpose as of 1955. Other material that has been published since seems to support the continued agreement on this as *the* principal purpose of college personnel work. It is interesting to note that the purpose includes two distinct elements. The one emphasizes the development of the individual to the limit of his capacities; the second emphasizes the recognition of each individual student to be accorded dignity because he is a human being. Neither of these aspects of the purpose is satisfactory when used alone. One implication of this central purpose is that the student personnel program or college guidance program has obligations to each individual in the student body. This suggests that the limited approach noted previously in my comments is inappropriate in terms of the general purpose of the field. Consideration of this purpose would also, I think, lead to criticism of the criterion of student satisfaction since it is not directly connected with the central purpose.

Using this conception of central purpose of college guidance work it is interesting to consider whether some criterion could be developed to evaluate such programs that would yield data allowing evaluation. It seems to me the only possibility of developing such data is that the criterion must describe some kind of behavior on the part of students: that such behavior must be closely related to the purposes outlined above; and that it must be sequentially observable in most if not all college students so that comparisons and distinctions can be made.

Several studies[8a] of "college student development" have recently been completed, but it is difficult to describe their meaning for research as to outcomes of college guidance programs since they lack conceptual relationships. The behavior of college students that seems to offer the most promising criteria for evaluation is the behavior of students as they make choices. The choice process seems to me to include more of the central purpose of the college guidance program than any other act of students. Choices concerning such factors as the curriculum, vocational and educational planning, and responses to social situations describe the individual human being as he is developing into maturity, and they have a very large ingredient of the individuality that seems so important to the guidance field.

In thinking about the ways in which choice-making decision could be developed into a criterion for the evaluation of college guidance programs, I should like to turn for a moment to the work that has been done in decision theory. The work thus far has been aimed principally at

studying the process of decision making as it is related to inspection and quality control in industrial production. The simpler problems that have been explored are decisions to accept or reject manufactured articles. An extension of decision theory into game theory adds a principle of uncertainty that comes from prospective selfish actions of competitors and, in the statistical sense, the uncertainty that comes from random variation of events. These studies of decisions take into account the risk, the benefit, or the utility of various forms of action. Experimental psychologists have recently begun to study how people actually choose between courses of action: that is, what utilities are consistent with their action.[9]

The major contribution of decision theory up to this point has been to illuminate the qualities of best decisions when the interests of many individuals are at stake as, for example, in the economic decisions of government.

The question we are concerned with has almost none of the dimensions of decisions that are best for large groups of individuals. Cronbach and Gleser,[10] in a recent exploration of psychological tests and decision theory, distinguish between what can be termed institutional decisions, which have to do with *best* choices for groups of individuals, and individual decisions, which have as their paramount interest the individual's desires and values. They point out that the qualities of these two types of decision theory are substantially different, and they note that current decision theory has been developed almost entirely in terms of institutional decisions and has not yet shed much light on the problem of individual decision.

It does seem possible to hypothesize that a student personnel program in a college might be evaluated in terms of the "goodness" of individual decisions by students. The problem in using classic decision theory is that the so-called pay-off functions (that is, utility) are not very applicable. What I propose is that it seems possible to describe the characteristics of "goodness" with respect to individual decisions by college students that are consistent with our cultural values and with more specific values in higher education. I therefore submit a tentative list of the characteristics on which one might test the "goodness" of individual decision by students:

(1) The use of meaningful informational items including expectancy and probability data derived from testing, and reality data in terms of planning vocationally and educationally would provide a scale by which one could characterize the "goodness" of individual decision by students. To put it simply, the student who, in making his individual decision, ignores informational items completely is, it seems to me, not making a good decision. On the other hand, if he relies on informational items completely to arrive at a decision it cannot then be said to have an individual quality.

(2) The willingness of the student to deal with ambiguity in the factors that lead to individual decisions on his part with no real test of outcomes external to his own value system and with no way to compare decisions made with those rejected as to utility would seem to me to be a measure of "goodness" of individual decision by college students. This might be said to be related to Allport's[11] comment that propriate strivings are usually directed toward those goals that are, strictly speaking, unobtainable by the individual. This recognition of uncertainty and lack of assurance of attainment is, it seems to me, consistent with the reality of the human situation in our society and with the purposes of higher education.

(3) "Goodness" of individual decisions by college students would, it seems to me, involve perception of the sequential nature of decision making. That is, at any one moment, a good decision would be one in which the individual is aware of the nature of his past choices, the possibilities of future choices, and the limitations of present choices, so that decision is always related to what has been, to what may be in the future, and to what is possible at the moment.

(4) Perhaps the most important characteristic that would determine the "goodness" of individual decision by college students would be the acceptance by the student of the recognition that individual decision is an expression of personal choice or preference as a major determinant. This, it seems to me, is related to the ability of the student to accept responsibility for his own acts and to recognize that his future to some extent lies in his own hands.

It has occurred to me that the notions of these concepts might not be as appropriate to develop criteria for the evaluation of high school guidance programs. There the students are of a younger age, more under the domination of their families and perhaps less able to make individual choices for which they are willing to accept responsibility. However, at the college age, such a conception for possible criteria seems to me to have considerable validity.

There will be those asking whether such a conception as I am proposing is not so vague that it will be useless to develop specific criteria for the evaluation of outcomes. My answer would be that any conception of a narrower range would provide inadequate measures of outcomes because it is not consistent with the agreed purposes of college guidance work; moreover such a conception does not prevent those whose particular responsibilities are more limited from measuring the outcomes of their particular functions in the particular college guidance program on a much more specific and limited basis. As I interpreted my task, it was to discuss the problem of criteria in the evaluation of college guidance programs. Such criteria, it seems to me, must have the characteristics of being broad enough to cover all elements of the pro-

gram and to measure outcomes in terms of most, if not all, students in a college or university.

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DIVISION OF MICROBIOLOGY

CELLULOSE ACETATE ELECTROPHORESIS IN MICROBIOLOGY AND IMMUNOLOGY*

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The utilization of oxoid cellulose acetate strips for electrophoresis stems from the pioneering researches of J. Kohn of Queen Mary's Hospital in Roehampton, England, who demonstrated that electrophoresis, when performed in this medium, could be accomplished in far less time and with greater sensitivity than had been feasible previously with paper or similar media (Kohn, 1957; Kohn, 1958*a*, 1958*b*).

The strips have a thickness of 130μ and a graded porosity through this thickness that ranges from 3 to 0.5μ. FIGURE 1 indicates a typical serum electrophoresis pattern performed in 2 hours and utilizing a specimen of 3 μl. Notice that the background is particularly clear, especially around the albumen and α_1 bands, and that there is splitting of the α_1 and α_2 bands as well as splitting of the β_1 and β_2 fractions. As little as 0.1 μl. of serum representing 3 to 5μg. of protein can be readily separated and stained using 0.002 per cent nigrosin. Recent papers by Grunbaum, Kirk and their colleagues (Grunbaum *et al.*, 1960; Atchley, 1960 Grunbaum and Kirk, 1960) have reported still greater sensitivity, whereby 0.01 to 0.1 μl. serum or blood samples or as little as 1.5γ horse spleen ferritin in a volume of 0.5 μl. have been successfully separated on oxoid strips after 45 min. electrophoresis.

Brackenridge (1960*a*, 1960*b*, 1960c) has reported the finest study to date on the kinetics of electrophoresis and staining with oxoid cellulose acetate. He showed that the dye uptake of Lissamine green SF by lower concentrations of serum fractions was independent of the staining time over the 25 to 150 min. period studied. Similarly, the dye uptake was independent of the staining temperatures examined from 10 to 30° C. Our own studies confirmed these findings and also showed that the intensity of staining with nigrosin was unchanged within a 4 to 18 hour period studied. Grunbaum *et al.* (1960), using oxoid strips and Ponceau S stain, have also made the observation that there was "no visible change in staining intensity between 2 min. and 1 hour." The advantages of these wide latitudes in temperature and time of staining are obvious.

Brakenridge also observed that all the stained serum protein fractions he studied, when eluted and quantitated by light absorption in a spectrophotometer, obeyed Beer's Law over a wide range of concentrations. Elution of the stain, however, is not readily feasible with the large num-

*This paper, illustrated with slides, was presented at a meeting of the Division on March 24, 1961.

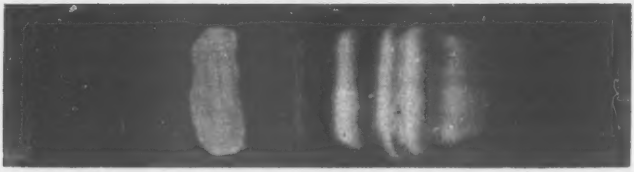

FIGURE 1. Electrophoresis on oxoid cellulose acetate using 3 μl human serum. Separation performed in a Shandon Universal Tank (Kohn design), 2 hours, *p*H 8.6 barbitone buffer, constant current of 1 mA/strip, starting voltage 200 v., stained with naphthalene black. (The image of this and the subsequent photos are reversed since the strip itself is readily used as a negative by being placed against a piece of photographic paper, light exposed, and developed without the need of direct photography).

ber of stains and reagents that may be used with cellulose acetate and my colleagues and I have therefore developed a technique for which the following experiment is illustrative.

(1) The electrophoretic run was made on 2.5 × 12 cm. oxoid cellulose acetate strips in a Kohn tank, using barbitone buffer, *p*H 8.6. Separate strips were charged with 1, 2, 3, and 4 μl of normal human sera.

(2) After 2 hours the strips were removed and dried for 20 min. at 100° C., stained 10 min. in 0.2 per cent Ponceau S in 3 per cent trichloroacetic acid, rinsed in distilled water, and washed 5 min. in 5 per cent acetic acid. Strips were redried for 10 min.

(3) Fractions (α_1, α_2, β_1 and γ) were cut out and each cut section dissolved in 2 ml. of a mixture of 90 per cent chloroform and 10 per cent ethanol. The albumen fraction and an entire strip for total protein determination were each dissolved in 4 ml. of the solvent (in the latter case the optical density (O.D.) values are therefore multiplied by 2).

(4) Readings were made in a Coleman Jr. Spectrophotometer using an 8 × 8 × 100 mm. cuvette and 520λ.

FIGURE 2 shows that all the serum components obey Beer's Law over the range of volume of sera samples used. Note that the sum of the O.D.s of all the components of the serum equals the O.D. of the total protein. The per cent of any fraction is easily computed:

$$\frac{\text{O.D. of serum fraction}}{\text{Sum of O.D. of serum fractions}} \times 100 = \text{per cent fraction}$$

Note also from FIGURE 3 that plotting the per cent serum fractions (per cent of dye uptake) against the O.D. results in a straight line curve, indicating that all the serum components take up the dye only in relation to their respective concentrations.

We have performed similar experiments with various other components such as cholesterol, amino acids, and alkaloids and, despite the wide variety of stains and indicator systems used, the method of quantitation

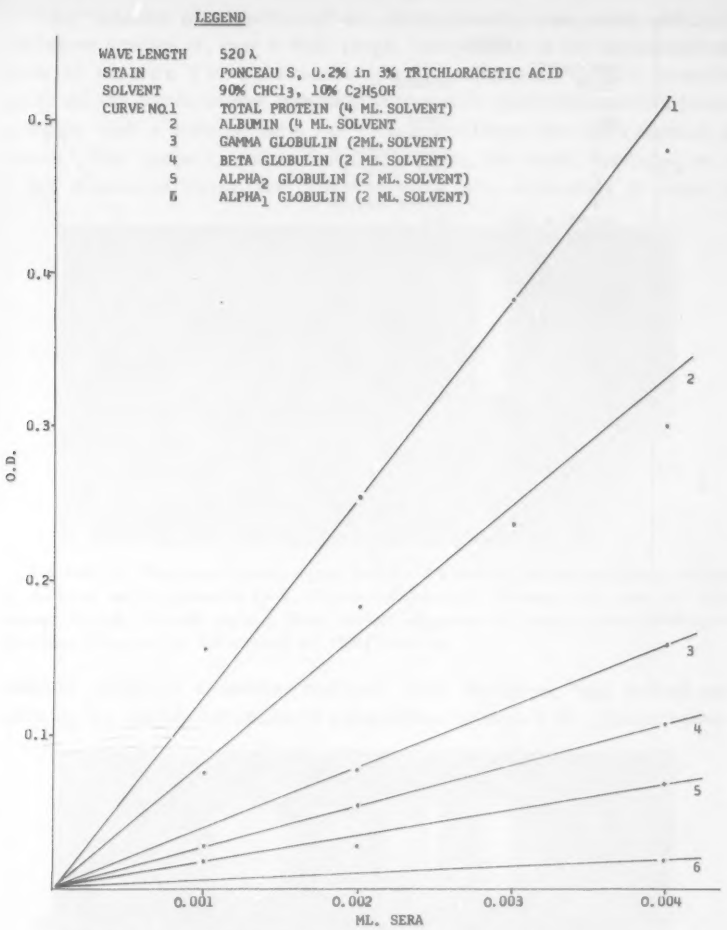

FIGURE 2. Normal human sera fractions quantitated by Coleman.

described here was applicable in every case. The significant advantages of this method for routine clinical as well as laboratory investigative work are:

(1) It is no more, if not less, time-consuming than quantitation by an automatic integrating scanner.

(2) No special expensive instruments need be purchased, since a spectrophotometer is readily available in most laboratories.

(3) The reproducibility is as good as and the sensitivity is superior to that attained with an automatic integrating scanner. Using nigrosin-

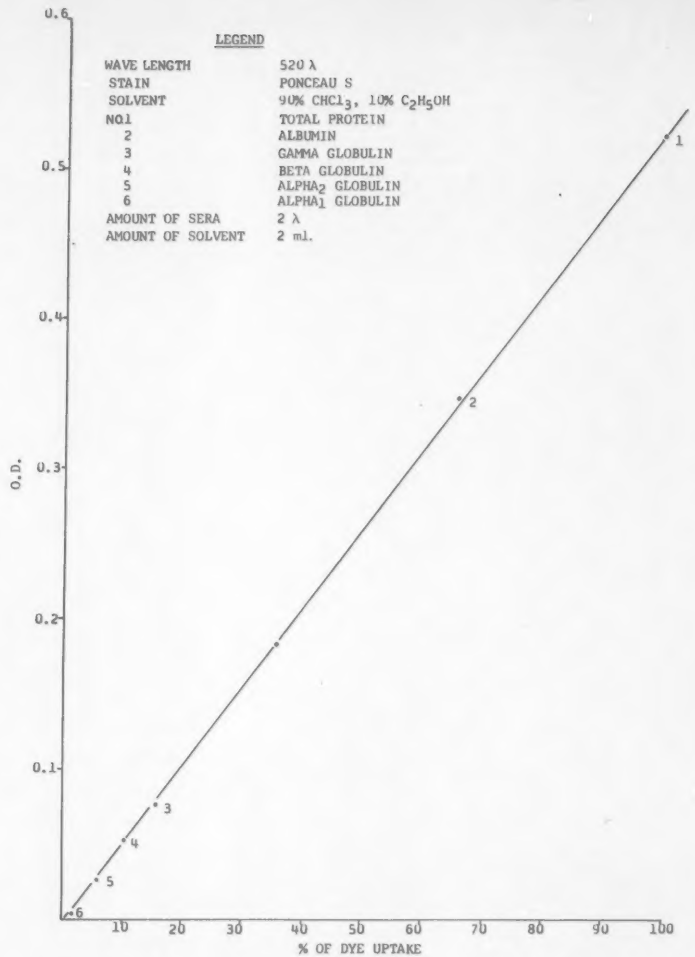

FIGURE 3. Normal human sera fractions quantitated by Coleman.

stained protein fractions and the ultramicro cuvette of the Coleman Spectrophotometer, one may quantitate as little as 0.03 to 0.05 γ of serum protein.

The necessary destruction of the strip using this method is not a serious drawback since it is routinely recommended that running of all specimens, especially of clinical material, be done in duplicate, and the use of a Vokam power unit coupled to two Kohn electrophoresis tanks will permit running of as many as 16 strips every 2 to 3 hours!

The velocity of migration of an electrophoretic component on oxoid cellulose acetate is, over a wide range, independent of its concentration. Note in FIGURE 4 that the serum sample, sufficiently diluted to extinguish all the bands except gamma globulin, still resulted in perfect juxtaposition with a matched strip run with seven times the concentration of serum. This property makes feasible running the same specimen on a large number of strips, and staining each strip separately in order to

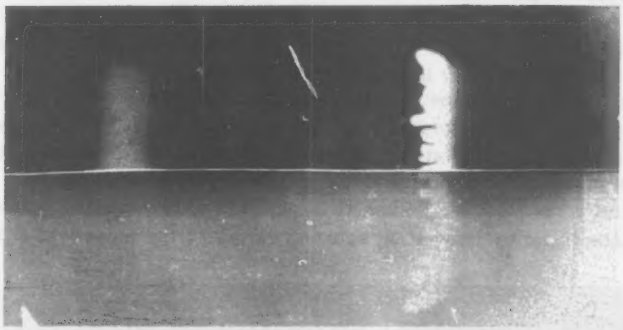

FIGURE 4. Abnormal human serum run for 2 hours on oxoid cellulose acetate in a Kohn electrophoresis tank, Ponceau-S stained. Bottom strip was run with serum diluted 7 × with saline. Note perfect alignment of gamma globulin despite fact that dilution has obliterated all other bands.

identify different molecular entities. This technique has proved applicable for the identification of lipoproteins (FIGURE 5), glycoproteins,

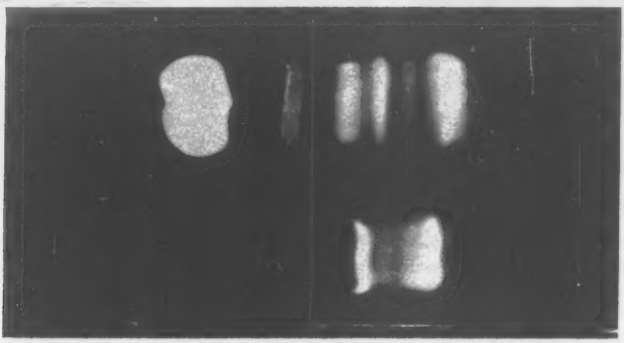

FIGURE 5. Two specimens of the same serum were run simultaneously. The top strip was stained with Ponceau-S. The bottom strip was stained for lipoprotein. With cellulose acetate it is unnecessary to stain for lipoprotein after electrophoresis. If Sudan black B is mixed with the specimen in a proportion of 5 parts Sudan stain to 1 part serum, the separation of lipoprotein bands may be directly observed during the electrophoresis run.

and identification and assay of normal and abnormal serum and tissue components (FIGURE 6).

A mixture of serum and an aqueous solution of polymyxin B was made so that 2 μl. contained 25 γ of polymyxin B. An aqueous solution of polymyxin B alone containing 25 γ in 2 μl. was used as the control. Barbitone acetate buffer was used but adjusted to *p*H 6.0 with acetic acid. The strips were charged at anode (this speeds up separation of polymyxin B from serum) and the Vokam power unit set at constant voltage of 100 V for 45 min. The strips were dried 20 min. at 100° C., stained 10 min. in light green, rinsed approximately 1 min. in distilled H_2O, and washed 5 min. in each of two 5 per cent HAc baths.

FIGURE 6 illustrates the separation of polymyxin B and the excellent juxtaposition for the serum as well as polymyxin B bands. The amount of antibiotic was quantitated from standard curves by dissolving the cut bands in a solvent and measuring the light absorption as described above for serum components. Values thus obtained were essentially identical to those found by integrating scanned curves of the polymyxin B bands.

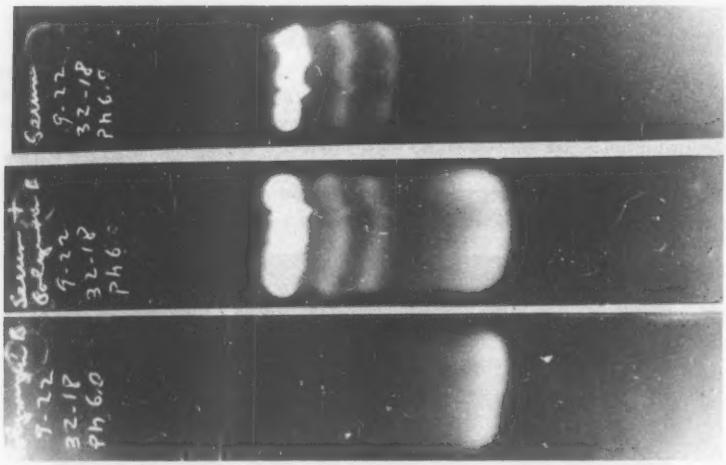

FIGURE 6. Top strip contains serum, middle strip contains serum with 25 γ polymyxin B per μ1 of serum, bottom strip contains same concentration polymyxin B alone. Electrophoresis in Kohn tank using constant voltage of 100 v, 45 min., and stained with light green SF.

FIGURES 7 and 8 indicate standard curves for known stained quantities of polymyxin B, showing a straight-line relationship from 1 to 60 γ of the antibiotic. Light green was used as a stain here for the sake of convenience of staining both the serum and the antibiotic, but more sensitive stains or color reactants would increase further the sensitivity of the analysis.

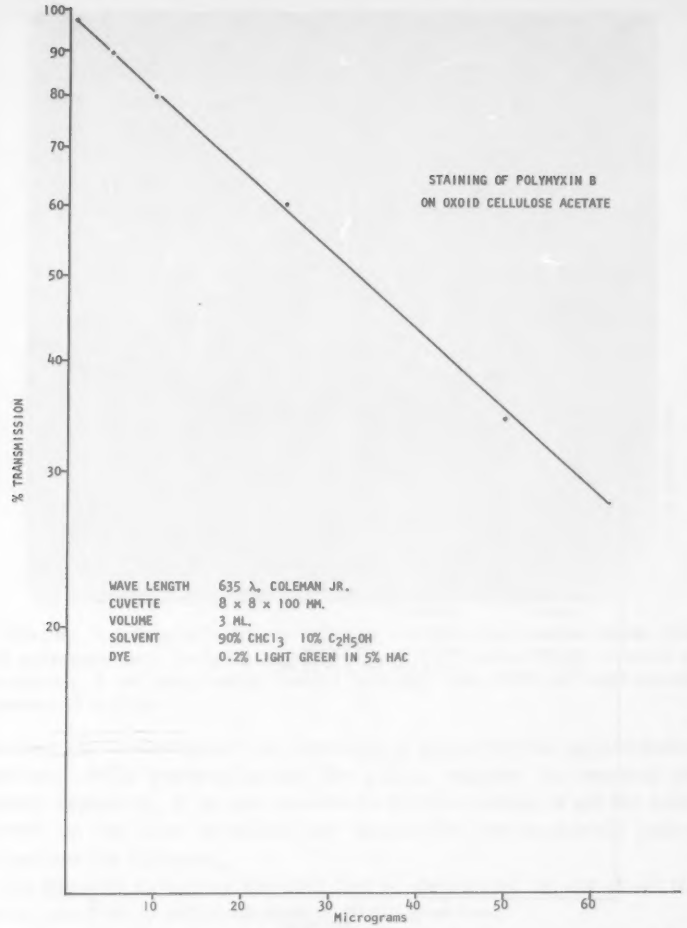

FIGURE 7.

This method of quantitating components that can be separated by electrophoresis and suitably color-reacted has been tested with substances of diverse chemical character and has proved uniformly successful.

Immunodiffusion Techniques

Immunodiffusion and immunoelectrophoretic techniques with oxoid cellulose acetate have been previously described (Consden and Kohn, 1959; Kohn, 1959; Kohn, 1960). The method proposed by Kohn utilized cellulose acetate membrane filters or sections cut from large strips. The

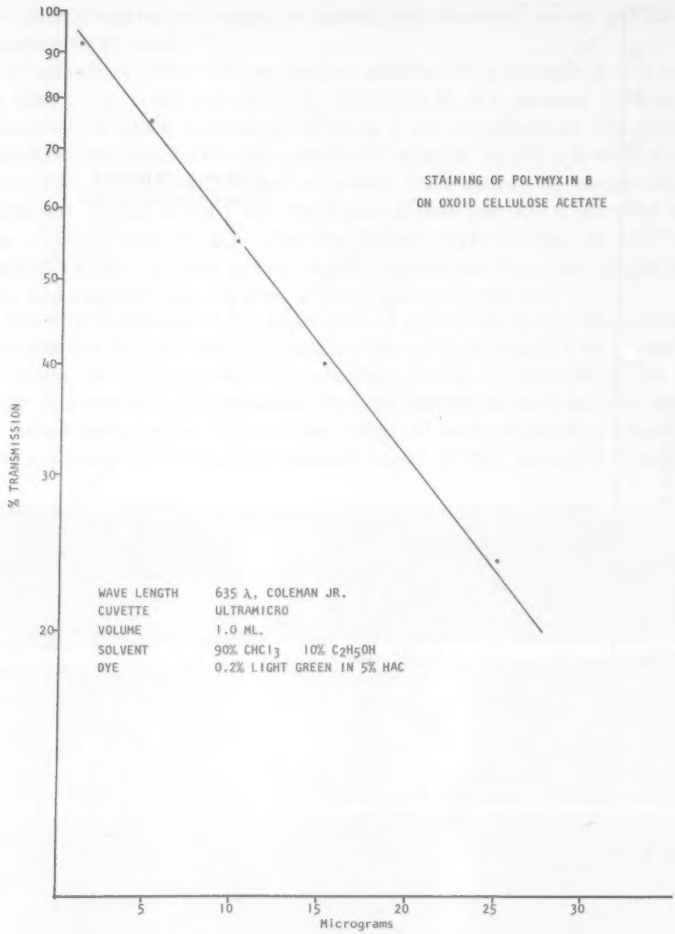

FIGURE 8.

antigen and antibody were placed on the buffer-wetted strip that was then immersed under Whitmore Oil No. 120. The precipitin bands are very sharp and conform in every way to conventional agar precipitin techniques in so far as interpretation is concerned (FIGURE 9).

The excellent juxtaposition of electrophoretically identical bands run on different strips makes possible the application of the following technique for immunodiffusion studies.

A slot is cut in a cellulose acetate strip with a razor blade or sharp scalpel and a straight edge, resulting in two sections as shown in

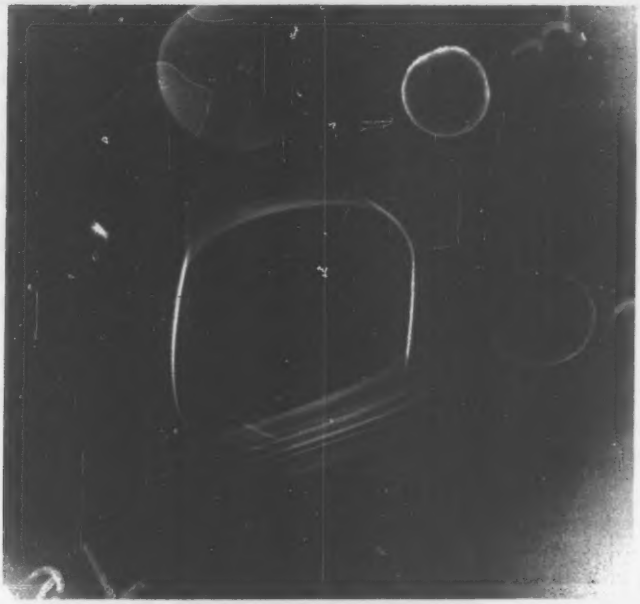

FIGURE 9. Immunodiffusion on cellulose acetate using various tissue fluids and antihuman sera. Antibody is in the center with various tissue extracts approximately 1 cm. from center. Stained overnight with 0.002 per cent nigrosin Courtesy of J. Kohn.

FIGURE 10. The material to be examined is placed on the top and bottom sections. After electrophoresis the bottom segment is removed and stained separately. It is now possible to fix the position of all the bands present on the upper unstained and undisturbed electrophoretic pattern and perform the following.

(1) Nitrogen values by Kjeldahl may be determined on any or all the bands, since oxoid cellulose acetate is nitrogen free.

(2) Discs of electrophoretically pure material may be punched from each of the bands with a small punch (FIGURE 10). These discs may be placed on buffer-impregnated cellulose acetate membranes, antisera droplets placed 1 cm. from the antigen-impregnated discs, and the membrane placed on a plastic pin-cushion type support in a closed moist chamber (submersion under oil as originally reported by Kohn is not necessary if a moisture saturated container is used). Handling after immunodiffusion is completed as previously described (FIGURE 11).

This technique may be used to determine immunological relationships of components from antisera that can be readily tested in all combinations by immunodiffusion against electrophoretic components of antigens.

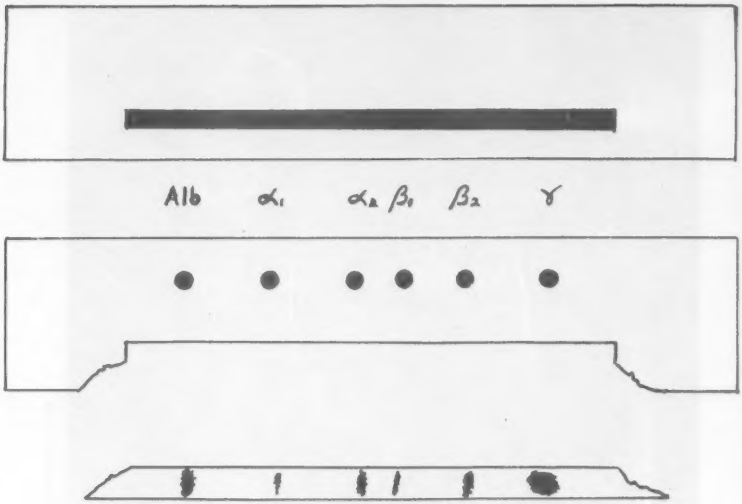

FIGURE 10.

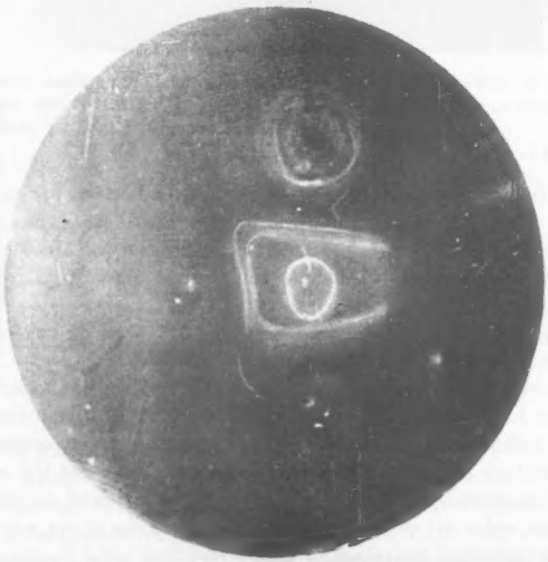

FIGURE 11. Antiserum against group A beta hemolytic streptococci is in the center. Cellulose acetate discs containing antigens prepared from streptococci lysed with Maxted enzyme were placed around the antiserum. Preparation of these punched discs is described in text. Precipitin bands were stained with 0.002 per cent nigrosin after overnight diffusion in a moist chamber.

Other Applications

Kohn has recently reported a convenient method of determining folic acid deficiency using ninhydrin and amino acid markers (personal communication).

J. E. Kempf of the University of Illinois, Chicago, Ill. (personal communication) has demonstrated the electrophoretic mobility of bacteriophage. It was of interest that the mortality of the phage following electrophoresis, as determined by plaque count, was relatively low.

We have performed electrophoresis of porphyrins in a Shandon (Kohn) tank using 0.1 *N* HCl as a buffer with excellent separation that could be readily followed under a UV light.

Summary

The properties of oxoid cellulose acetate strips for electrophoresis and immunodiffusion techniques are described. Their unusual sensitivity as well as speed of operation and clarity of separation provide a new tool where electrophoretic techniques are applicable.

Acknowledgment

The most capable technical assistance of LaVerne Decker is gratefully acknowledged.

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DIVISION OF ANTHROPOLOGY

PROBLEMS IN THE ORIGINS OF THE CIVILIZATIONS OF INDIA AND CHINA*

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Study of the early civilizations of western Asia reveals that there are essential differences between the Egyptian civilization and the Sumerian. This dichotomy in primary civilization can be best expressed by listing some of the contrasts (TABLE 1).

Although these differences must be qualified both by reason of local emphases, that is, within the culture and by chronological change, the form of the civilizations as expressed by these and other traits is in each case markedly dissimilar.

There is general agreement that the Sumerian civilization has some chronological priority over the Egyptian and that, in fact, at least some of the impetus to a civilized state in the Nile Valley came from Mesopotamia in predynastic times. Sumerian civilization was an indigenous development, the final phase of a cultural evolvement that started with the onset of food production. Stages in this evolution have been archaeologically traced. These stages evidence: (1) a centripetal movement, beginning with initially numerous scattered dwelling units, then fewer but larger villages and, finally, a town-to-city growth; (2) an increasing number of specializations including those not directly involved with food production; (3) development of monumental building; (4) indications of an augmenting foreign trade; (5) public projects needing administration; (6) a mutually intelligible symbolization approaching the use of abstract signs; (7) the multiplication of protocities and the diminution of the number of villages; (8) the development of formal government to handle the increasing complexities of population concentration; and (9) an increasing contact and actual conflict with neighboring populations.

The end result of this development is urbanization which, in Sumeria, placed the majority of the population in or near cities. The evidence proves that city life, with its emphasis on human interaction on a large and complex scale, brought about accelerating idea-and-technique exchange. This was the prime reason for the specific dynamic character of Mesopotamian civilization.

The development of civilization in Egypt is blurred somewhat by a lack of stratigraphic evidence. It is clear that once food production took hold in the Nile Valley there was an indigenous development in a stage of considerable isolation. Egyptian prehistoric cultures evidence a slower change from hunting to full food production than in Sumeria; population

* This paper, illustrated with slides, was presented at a meeting of the Division on March 27, 1961.

TABLE 1

	Egypt	Sumer
Art style	Conservative	Changing
Architecture	Sacred	Secular-sacred
King	God	God-influenced
Court	Landed aristocracy	Elders
Writing	Hieroglyphic-hieratic cursive	Cuniform abstract
Technology	Traditional	Inventive
Texts	Sacred	Sacred and business
Thought	Nonspeculative	Speculative
Public works	Pyramids, temples	Temples, irrigation
Locale	Isolated	Vulnerable to outside
Majority of population	Rural	Urban

increase was expressed by the multiplication of villages rather than the growth of major centers; craft styles are long-lived, and crafts themselves multiply less rapidly – although fine stone vases, for example, indicate a high level of achievement. The enormous extent of the Egyptian Nile, including the delta, may have been a significant reason for the localization apparent in the predynastic period. Boats, however, were used in Amratian Times, and the spread of craft styles and other such developments was via river trade. Gravitation toward certain centers for ceremonial purposes and, perhaps, for military concentration appears to have already begun in the Amratian. These centers were apparently large villages or perhaps towns visited only seasonally by people who lived in villages throughout each district. Certain symbols of animals, birds, and objects that occur in vase painting were probably totemic, representing a tribe or clan that lived in a particular district. These symbols may have been the basis for the district or nome divisions of dynastic times. Authorities generally agree that brick building came to Egypt in the Late Gerzean or First Dynasty and motivated the building of monumental structures. Cylinder seals, art motifs and, very probably, pictographic writing accompanied what is clearly a strong Mesopotamian influx of invention and idea. This Mesopotamian influence appears to have accelerated Egyptian technology and begun the formalization of both religion and the social and political order. However, the diffuse settlement pattern of the Egyptians was not affected and only one center perhaps (that is, Memphis) can be said to have urbanized.

The homogeneity of Egyptian civilization can be said to be a product of the Nile that provided the means for rapid and efficient communication. At the same time, Egypt's geographical isolation contained the tendency to spread, which was the natural result of growing population and improved techniques of food production. Political control of Egypt, expedited by the Nile highway, began as a result of district rivalries and was enlarged by alliances and conquests. Once achieved, the state acknowledged local rulers and feudal control was manifest. Local allegiances of villages

within districts were probably maintained by traditional social controls. These were very likely extended to the pharaonic institution perhaps in a fashion similar to that still extant among certain tribes in Africa today (for example, among the Bemba of Rhodesia). The ostensible national form of Egyptian civilization is deceptive in that it suggests an urban complexity by definition. In Egypt the population was widely spread. There were local alliances and social forms that differed but slightly from neighboring localities but commanded the participation of each villager. The pharaonic intrusion upon this local structure was more than likely formal and prescribed rather than arbitrary. The conservative and traditional style of Egyptian traits owes its origin to this rural economic and social configuration.

Clearly we have in the ancient Near East two specifically different kinds of civilization. In form, the Sumerian is concentrated and dynamic; the Egyptian, diffuse and stable. Historically the Sumerian form can be traced in the Greek "polis" system, the Roman state, the Renaissance city and, in fact, to modern, that is, Western civilization. Because this form of civilization is so inclusive, I propose to call it the SU-civilization, the letters standing for its earliest manifestation. In contrast, the EG-civilization pertains to that form outlined for Egypt.

We are now in a position to examine the origins of the civilizations of India and China in the light of these Near Eastern aspects of civilization. Evidence is now virtually conclusive that there is a chronological reason, among other reasons, for the priority of Near Eastern civilization over those of the east. In fact the evidence now indicates that the Harappan civilization came into being after 2300 B.C. and the Shang after 1700 B.C. if not somewhat later. Mortimer Wheeler has acknowledged India's civilized debt by stating that the "idea of civilization" came to the Indus Valley from the west. The term "idea of civilization" has also been used for China by several authorities. The implication of this term is that civilization is an entity in itself that is carried entire to another region where it is interpreted into local styles and thus appears on the scene unique and similar in one paradoxical lump.

The factors that bring about urbanization and at the same time give a specific form to the urbanization are complex. One thing is obvious: there has to be a considerable local prelude to civilization to bring about its specific styles.* The accumulating evidence for the movement of the various phases of food production eastward across Asia gives us that assurance that the necessary prelude to early civilization was established in many regions by the Third Millennium B.C. In studying this movement, three features are outstanding: (1) the movement was that of a grain-growing agriculture (millet, wheat, barley) that had its geographical

*I have used style and form interchangeably throughout. See Kroeber, 1957; Frankfort, 1951.

limits fairly prescribed to an area south of about the 45-degree parallel and outside the tropical forest region of India, China, and Southeast Asia; (2) in areas where the movement can be followed, a spurt in development occurred with the use of cattle as draught animals; and (3) there was a multiplication in number or growth in size of villages wherever settlement was possible.

In other papers I have attempted to trace the development of village cultures in the Indo-Iranian borderlands (Fairservis, 1959, 1961). Various reasons have been given to prove that there was a parallel development in the Indus Valley with that of Baluchistan. Geographical factors prevented the final fruition of the Baluch cultures into civilization, but these reasons were not operative in the Indus Valley, and civilization came about there. The evidence would indicate that the village cultures of Baluchistan developed numerous special crafts, and thus nonfood-productive specialists were resident. Seals and potters' marks show mutually intelligible symbolizations; brick structures of considerable size mark formal and monumental building and, together with figurines and sacrifice, argue for formal religious practice.

The Harappan civilization is characterized by an enormous geographical spread into the eastern Punjab and as far south at least as the Narbada. The latest phases of the culture occur outside the Indus Valley generally and the earliest within it, especially in Sind. The majority of the now over 100 known sites are villages, but there are perhaps 4 or 5 large urban centers, of which Harappa and Mohenjo-Daro are the most famous. The upper levels at these latter sites also represent late phases of the culture.

Study of the material culture of the Harappan civilization reveals a remarkable homogeneity of style in both time and space. There is no essential difference in the form of writing, ceramic decoration, and building structure, for example, as they are represented at Mohenjo-Daro in the mature stage when compared with these features as found at Lothal in Gujarat or even as known at Alamgirpur near Delhi. In the stratified sites where phases of the Harappan culture can be determined, trait evolution indicates a deterioration of style from early to late with very little innovation. One gains the impression of a rural culture existing in isolation, with traditional styles and rigidly prescribed social forms governing the manner of life. In character, then, the Harappan civilization is of the EG-civilization type.

Mortimer Wheeler has noted that the "idea of civilization" diffused to the Indus Valley (Wheeler, 1959, 104). The disseminator was, of course, Sumeria, the nearest civilized neighbor. In spite of the occasional finds of Harappan seals in Mesopotamia, it does seem on the present evidence that contact between the two areas was extremely casual and very rarely direct. The Mesopotamian contribution appears to have been the ziggurat-type structure for ceremonial purposes, the wheeled cart, certain icono-

graphic and art design elements, and some pottery, bead, and other craft forms. The problem of the writing is still not solved. It appears to be pictographic or at least hieroglyphic. It has no relationship with cuneiform and its antecedents are unknown unless potters' marks, such as those found in the Ouetta Valley, are indicative of one stage of development (Fairservis, 1956, Pl. 14). However, this would imply an evolvement from the abstract symbol to the pictographic: an unlikely happening in view of the progression elsewhere. On the other hand, these potters' marks were unquestionably mutually understood, and their motivation may well have derived from contacts with the writing system of Mesopotamia. Thus they would be the medium by which the "idea of writing" was conveyed to the Indus Valley. This process could have begun in pre-Sumerian (or proto-Sumerian) times however.

The settlement pattern of the Harappan civilization appears to have consisted of a clustering of small villages around a large center; the number of villages thinning out the farther from the center (Fairservis, 1961). Village sites, in fact, occur at considerable distances from these centers. The cities, as we may call these centers, were regular in plan: an elevated area crowned with formal structures, including temples, storage places, and ritual tanks; and a low area with residences either surrounding the formal area or to one side of it. Fired brick, drainage channels, and regularity of street and building plans are characteristic features. The city itself is close to the river, and evidence of frequent flooding and rebuilding is abundant. The city as a whole shows a traditional and formal context with less emphasis upon the secular and more upon the sacred. It seems clear on the present evidence that the Indus Valley cities at least were ceremonial centers. In contrast to Sumeria, whence the idea of elevating sacred buildings probably derived, there does not seem to have been a conjunctive political control exercised from these cities.

The centralizing factor appears to have been religion. There are no indications of irrigation, and the major public work seems to have been the brick buildings and protective dams in the urban centers. The village orientation of the populace is now implicit in our evidence. There is evidence that religious shrines existed in the villages. The ceremonial center would, therefore, be an extension of the village pattern.

If we list some of the principal traits of the Harappan civilization, we discover that a majority of them apparently derived from older village cultures of the developed food-production stage as known on the Iranian plateau or in the pre-Harappan cultures of the Indus (TABLE 2).

Food production can be said to contain the seeds of civilization within itself. The trend of food-producing cultures is toward centralization, elaboration, and specialization. Whether this is entirely due to stimulus diffusion, or to a kind of natural law is, of course, impossible to say. The fact that civilization developed in the Indus Valley and not on the

TABLE 2

Harappan	Origin
City	DFP (developed food production), village ext.
Writing	Mesopotamia
Elevated temples	Mesopotamia
Pottery forms	DFP (greatest per cent)
Pottery designs	DFP (greatest per cent)
Cattle domestication	DFP
Human and animal figurines	DFP
Stone cutting and polishing	DFP
Priest hierarchy	DFP
Brick	DFP
Fertility cult	DFP
Wheeled vehicles	Mesopotamia
Weaving	DFP
Weights and measures	Mesopotamia (?)
Town planning	Indigenous
Dams	DFP
Canals (?)	Mesopotamia
Drains	Indigenous
Baths	Indigenous
Seals – square	DFP
Inlay	Mesopotamia
Copper use	DFP

Iranian Plateau may be accounted for by the symbiosis of men and cattle described in another paper (Fairservis, 1961). The quantity of energy contributed by cattle is significant when there is sufficient fertile land to muster their power effectively. Studies in this field are only beginning.

Our evidence thus far would indicate that Indian civilization was the direct result of advantages given by evolving food production. Direct Mesopotamian influences were few and not necessary to create civilization. The form of the civilization is EG-civilization and, in fact, the development of Harappan civilization is not unlike that of Egypt.

Soviet researches in Central Asia have extended the distribution of Iranian type prehistoric village cultures up to Ferghana on the western flanks of the Altai Ranges. On the east in Chinese Turkistan there has been so little systematic survey that the evidence at the present time is inconclusive. However, early village sites have been found in some continuity from western Kansu eastward to the Yellow Sea, particularly in the drainage area of the middle and lower Huang Ho. As long ago as 1923, Anderson pointed out parallels between the painted pottery of North China (Yang Shao) and that of Anau. Subsequently Lauristan Ward, Heine-Geldern, and others have shown the close parallels between the gray pottery cultures of northeastern Iran and vicinity and those of the Lung-shan or black-pottery cultures that in places succeed the Yang Shao of the Yellow River plain. The evidence is sufficient to confirm the fact that western grain production reached North China and with it some of the cultural traits familiar in western Asia. A date of 2500 B.C. (or some-

what earlier) for the beginning of this settlement in North China seems warranted by our evidence.

In view of what we know of the genesis of civilization in India and in Egypt, we should expect that the potential for civilization was achieved when food production reached the North China plain. The stimulus of early Mesopotamian civilization so far afield, if it diffused via the chain of food-production cultures extending eastward, can be expected to be very weak. Direct contact is out of the question on the present evidence.

Neolithic China, as represented by the Yang Shao and Lung-shan cultures and their expression in Kansu and elsewhere, has some specifically western traits. Among these are painted pottery, clay bangles, clay stoppers, grinding stones, flexed burials, and grain agriculture. Assuming that the same motivations for a writing form existed in Neolithic China as they did in India, we can assume that some method of mutually intelligible symbolization was instigated by western food production as it developed in North China.

There is no evidence for irrigation on the Yellow River plain either in the Neolithic or Shang Dynasty and, in fact, it seems certain that rainfall was the prime moisture source for cultivation in North China. The sites are widely scattered and there is no indication as yet that proximity to the river was prerequisite for agriculture. Quite the contrary: it would appear that developing agriculture permitted the spread of settlement away from rivers. There is ample evidence that a strong North Asiatic element was present in the Chinese neolithic. This has a number of exemplifications: the semilunar knife, polished stone axes, semisubterranean houses, fire pits, textured pottery, harpoons, net sinkers, and fishhooks are among these. Lauristan Ward has already postulated a hypothetical early neolithic culture for China that would contain some of these North Asiatic traits but has as yet evaded the exploring eyes of the archaeologist. The Chinese neolithic cultures that have been encountered indicate that fishing and shellfish gathering were always important to the economy. Thus it is more than likely that, as in Siberia and in the Jomon of Japan, there was a considerable concentration of people along the coasts and shores of the rivers (sites now obscured by river and shore changes). With agriculture the vast loess plain of North China was opened to economic advantage, and a kind of decentralization occurred.

Neolithic China is given its special character by the blending of these two main influences. There are, however, indigenous features that also mark the cultures as Chinese. Among these are characteristic pottery forms such as the Li-tripod and the Hsien cooking pot, effigy-headed vessels, jade or fine-stone rings, and the stone hoe. It would appear that the extension of grain agricultural communities toward the Yangtze Valley let to experimentation with rice growing and, perhaps, with sericulture: two characteristically Chinese features.

The sequence of pre-Shang cultures in North China is still somewhat puzzling. Where they were in contact, the Yang Shao is earliest, the Lung-shan following, and the so-called Hsiao-Tun (gray or beaten pottery culture) the latest. However, they appear to be regionalized and, at the same time, they share so many traits that they express both contemporaneity and exceedingly slow evolvement. Critical to these researches is the fact that although sizable communities were established during periods represented by these cultures, there is *no concrete evidence* that the latest phases were at a level of protocivilization in the same way as were the Gerzean, Protoliterate, or Amri-Kulli cultures. The archaeological evidence does not as yet confirm the oft-reiterated Chinese idea that their civilization's origins were self contained.

The first civilized manifestation in North China is that of the Late Shang Dynasty. This is a surprisingly hybrid civilization, characterized by a very few urban centers where resided a warrior group ruling a largely decentralized rural village settlement. At Anyang the separation of the city into sections – each for a special class of artisans – gives a hint as to the kind of city that first appears in China, a political capital aristocratic in aspect. The EG-civilization assignment for Shang China is obvious in that the vast bulk of the people lived in villages and traditional ways were maintained in that conservative atmosphere. The Shang city with its aristocratic aura was also a religious center, as the oracle writings and the formality of remains indicate. The great Shang tombs emphasize the divinity of the monarch.

Examination of the traits of the Shang Dynasty reveals a strong Central Asian element, evidenced by wheeled chariots, horse domestication, sacrificial ritual of a characteristic kind, compound and reflex bows, hints of a solar faith rather than one emphasizing earth fertility alone, horse burial, retainer sacrifice, and extensive working in metal. The crafts of the Shang also reflect the older basic Chinese culture in that pottery, stone, and bone artifact forms are continued. Shang bronzes are important in that they are based on the older pottery forms, preserving at the same time remarkable decorative patterns and ornamentations that probably existed, in Neolithic China, only in perishable wood. These designs, also apparent in the stone sculpture of the Shang, are evidence for a North Asiatic trait complex in the earlier culture.

The Shang civilization is different from the other earlier civilizations of Asia in that it was later in appearing (*ca.* post-1700 B.C.) and was not apparently brought to fruition indigenously in the same way as in Egypt and India. Central Asia may be regarded as the cause of China's rise to civilization. All our evidence indicates that the horse played a major role in this. The rise of communication between vastly separate geographical regions that the horse permitted certainly accelerated the transference of traits from one group to another. Thus it seems likely that

some Mongoloid group, perhaps of the Mongolian region, adopted traits in common use in western Central Asia brought previously from the Near East, and moved into a region long settled by Neolithic Chinese. The result was not only an influx of new traits into China but a stimulation of old and thus a civilized culmination. This process reoccurs later, with both the Chou and Ch'in dynasties as good examples.

In summary, it is obvious that the rise to civilization is not identical in each area where civilization occurred, nor is the character of civilization necessarily similar because of proximity. The Sumerian civilization was the result of an indigenous development. The Egyptian was also largely indigenous but crystallized by strong Sumerian influences in its formative period. The Sumerian civilization was city-oriented and, therefore, dynamic and unstable; as such it became the root of Western civilization, the Egyptian was village-oriented and thereby became traditional and conservative. India's rise to civilization was largely the result of the wholesale development of food-producing cultures on the Iranian Plateau. This movement into the Indus Valley removed geographical restrictions and thus brought about the village-oriented, religion-permeated, and thus EG-civilizationlike Harappan civilization. Mesopotamian influences may have helped somewhat in this development but were not as important as has been regarded previously. China's civilization was based on extraordinarily disparate influences that combined to produce the characteristic style of that civilization. Western food-producing cultural influences on an indigenous population, largely North Asiatic in cultural derivation at least, brought about Neolithic China, which was featured by a widely dispersed village settlement, primarily on the North China plain. There is no rapid tendency toward urbanization apparent in the archeological evidence so far. However, the influx of Western Asiatic civilizing traits, via Central Asian forms and influences, caused an urbanization characteristic of the EG-civilization.

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DIVISION OF BIOCHEMISTRY

SOME ASPECTS OF COLLAGENOLYTIC ACTION*

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Collagenases are specific proteinases that catalyze the hydrolytic cleavage of undenatured as well as denatured collagens. Since many proteolytic enzymes including trypsin readily digest various preparations of gelatin (the common denatured form of collagen), the *sine qua non* for collagenase action is reactivity toward the undenatured substrate. Collagenases are known to occur extracellularly in media supporting the growth of several species of *Clostridia*,[1-3] and have been reported in larvae of blowflies. The enzyme used in our studies is that obtained from *Clostridium histolyticum*.

In recent years interest in collagenase has been fourfold. First, its action as a bacterial "endotoxin" contributing to the pathogenicity of clostridial organisms; second, its possible use in medicine and surgery as an agent promoting debridement of injured tissues; third, its unusual characteristics as a proteolytic enzyme with specificity for regions of protein molecules rich in glycylprolyl bonds that make it highly specific for collagens and gelatins; and, fourth – corollary to the last mentioned interest – its use to probe the structure of collagen.

Assays for collagenase depend on the nature of the substrate selected and the method used for detecting an end point of collagenolytic action. The primary standard method must be one that employs an undenatured protein substrate, or a peptide of amino acid sequence uniquely attacked by collagenase if a synthetic substrate is used. Since the specificity of collagenase has been studied only for the pure enzyme obtained from *C. histolyticum*, a synthetic substrate of the nature proposed by workers in Grassmann's laboratory[3] may be suitable for this enzyme alone; furthermore, it may not distinguish between collagenase and certain "broad-spectrum" proteolytic enzymes of, for example, molds.

For assay, the substrate we have chosen is the purified acid-soluble collagen of carp swim bladder (ichthyocol) or of calfskin. Assays are conducted using a uniform suspension of the substrate in dilute buffer

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The Division of Oceanography and Meteorology held a meeting on March 28, 1961, at which Leonard Greenburg of the Albert Einstein College of Medicine, New York, N. Y., presented a paper entitled "Meteorology, Air Pollution, and Health." This paper will not be published by the Academy.

at neutral pH, in which case the collagen becomes solubilized by enzymatic action, and progress of proteolysis may be monitored by examination of suitable filtrates with either ninhydrin or Folin (phenol) reagents.[4] In addition, a viscometric assay has been developed,[4] based on the fact that solutions of ichthyocol in 0.5 *M* calcium chloride have high viscosities that diminish rapidly as collagenolysis proceeds. We have defined a unit of collagenase as the amount of enzyme that results in the solution of 1 mg. of collagen (ichthyocol) under conditions of the suspension assay. A conversion factor has been determined experimentally that makes possible the expression of activity obtained in a viscometric measurement in terms of units defined by the suspension assay.

The enzyme has been purified by a combination of ammonium sulfate fractionation and treatment with alumina gel, and a preparation of high specific activity obtained (130 U./mg. of protein).[5] Preparations of similar order of activity have been obtained by Grant and Alburn[6] by fractionation on columns of modified cellulose. The purified enzyme has the characteristics of a globular protein of molecular weight approximately 109,000, a sedimentation constant of 5.4 S, and diffusion constant of 4.3×10^{-7} cm.^{2}/sec.[5] It requires the presence of calcium ions for full activity against its protein substrates, and is inhibited by cysteine, mercapatoethanol, and other sulfhydryl-containing agents. It is also inhibited strongly by metal-sequestering compounds such as *o*-phenanthroline, 8-hydroxyquinoline and α,α'-dipyridyl. From the action of such inhibitors, and from the fact that sulfhydryl compounds do not cause observable cleavage of the enzyme, it has been concluded that collagenase contains a metal, perhaps zinc or iron, as an intrinsic part of the molecule.

The specificity of collagenase has been studied extensively in recent years. The first substantial clues as to the nature of the bonds cleaved were obtained from studies of the peptides resulting from action of the enzyme on purified collagens. Experiments in our laboratory showed that the average molecular weight of such peptides was 500 to 600[8], and that nearly all contained *N*-terminal glycine while exhibiting no specificity with regard to the *C*-terminal residue.[7] We found also,[8] as did Schrohenloher *et al.*[9] that two peptides predominated in a collagenolytic digest of collagen: Gly-Pro-Ala and Gly-Pro-HyPro. Recently, Grassmann and his co-workers[10] suggested that a third peptide beginning with the sequence Gly-Pro-Gly- was also an important product of collagenolysis. Thus it was concluded that one requirement, in a substrate, for the action of collagenase is a sequence containing -X-Gly-Pro-Y-, in which X and Y are any of a number of amino acids in collagen. Experimental results of Nagai and Noda,[11] Heyns and Legler,[12] and Kasakova *et al.*[13] were in accord with this requirement and suggested that, in synthetic substrates, -Pro-X-Gly-Pro-Y- was an even more favorable sequence. It

may be said that Pro penultimate to X is enhancing, but not obligatory, for the action of the enzyme.

The first synthetic substrates found for collagenase were a series of co-polymers glycine proline furnished by E. Katchalski and A. Berger of the Weizmann Institute of Science, Rehovoth, Israel, and studied by Seifter *et al.*[5] A sequence polymer of Pro-Leu-Gly was digested by collagenase as described by Nagai and Noda;[11] and many simpler peptides (for example, Gly-Pro-Leu-Gly-Pro-NH_2 and carbobenzoxy-Pro-Ala-Gly-Pro-NH_2) were also digested. All substrates cleaved by the enzyme resulted in peptides containing *N*-terminal glycine in accordance with the predicted specificity. Recently, using compounds prepared by Y. Wolman of the Weizmann Institute, we have shown that the repeating sequence polymers, $(\text{-Pro-Ala-Gly-})_n$, $(\text{-Pro-Gly-Gly-})_n$ and $(\text{-Pro-Gly-HyPro-O-acetyl})_n$ are cleaved by collagenase. When tested in Tris buffer (*p*H 8) in presence of calcium ions, using a substrate concentration of 1 mg./ml. and an enzyme concentration of 5 U./ml., these compounds underwent maximum hydrolysis when incubated overnight at 37° C. Half-times for enzymatic hydrolysis were 4, 6, and 26 min. respectively for the polymers in the order given. The *n* value for the compounds is not known with certainty but is in the range of 4 to 6.

It is not altogether certain that calcium ions are required for enzymatic activity when synthetic substrates are used, and there are data indicating that cysteine does not inhibit collagenase acting against certain synthetic substrates as it does when collagen or gelatin is the substrate.[12]

The possibility that proteins other than collagen may be split by collagenase is not remote since the -Gly-Pro- sequence is found in several proteins including the beta form of the melanocyte stimulating hormone, and tobacco mosaic virus protein. In all these cases it is true that the second Pro residue that seems to enhance collagenase activity is not present. Preliminary evidence, however, seems to indicate that the C-terminal sequence of tobacco mosaic virus protein, -Gly-Pro-Ala-Thr, may be removed by action of collagenase; this observation is still to be confirmed.

It is of interest that collagenase digests its protein substrates at rates 10 to 100 times greater than those with which it hydrolyzes model synthetic substrates. Also worthy of note is the fact that the enzyme is approximately 10 to 12 times more active on gelatin derived from ichthyocol than on undenatured ichthyocol. Finally, it acts much more rapidly on undenatured ichthyocol than on undenatured acid-soluble collagen of calfskin. Thus, with regard to protein substrates, collagenase is most effective against denatured forms and least effective against native, highly cross-linked forms.

The fact that the enzyme acts on both denatured and undenatured species of its protein substrate offers a useful tool for studying the

effects of secondary and tertiary structures on proteolytic activity. Thus the papers of von Hippel and his co-workers[14,15] demonstrate that the kinetics of collagenase on ichthyocol in solution can be reduced to the sum of two concurrent reactions, both apparently first-order in substrate concentration but differing markedly in rate. The complex kinetics may reflect local differences in configuration in the vicinity of the susceptible peptide bonds. In contrast the kinetics of collagenase action on gelatin can be ascribed to a single apparent first-order reaction.

Furthermore, the action of the enzyme on collagen and gelatin existing in solution at the same time has been studied.[5] Calf-skin collagen in 1.0 *M* calcium chloride was mixed with varying amounts of the same solution in which the collagen had been gelatinized by mild heat; for the series of mixtures tested the *total* amount of protein substrate was constant in each tube. To each tube was then added collagenase, and the change in viscosity of the mixture was measured with time; the results, properly interpreted, therefore indicated progress of proteolysis of undenatured substrate since the viscosity of collagen is so much larger than that of gelatin. At the same time the total enzymatic activity (on collagen *and* gelatin) was determined by use of a ninhydrin procedure. It was found that the rate at which collagen was digested was dependent on the concentration of co-existent denatured collagen, that is, action on collagen decreased proportionately to the ratio of gelatin to collagen. The data seemed to fit the case of two equally competitive substrates for an enzyme; however, since the over-all reaction rates were quite different, they indicated that the peptide bonds in gelatin were more easily severed than those in collagen. It would seem that the enzyme binds to a substrate molecule and remains with it until proteolysis is complete, then disengages and moves to another substrate molecule. Since proteolysis (the action subsequent to binding) is more rapid for gelatin than for collagen, the kinetics of enzyme action on a mixture of the substrates always follows an intermediate course at first and then drops off to a course characteristic of the digestion of collagen.

Returning now to the nature of the products of hydrolysis of either collagen or gelatin acted on by collagenase, one notes that no free amino acids result. However, the peptides formed can be further degraded by the action of peptidases with release of free amino acids. Thus TABLE 1 shows the results of action of swine kidney leucine aminopeptidase and prolidase (imidodipeptidase) on a digest of gelatin derived from ichthyocol. Amino acids were determined by ion exchange chromatography according to the method of Moore and Stein using a Spinco automatic analyzer. It may be seen from the results that imidodipeptidase acted on collagenase digest to release few free amino acids (3.8 per cent of the total glycine and 1.9 per cent of the total alanine), and this suggests that its sole action was further cleavage of a small amount of tripeptides. Leucine aminopeptidase, however, caused appearance of significant

TABLE 1

FREE-AMINO ACIDS RELEASED FROM ICHTHYOCOL GELATIN AFTER DIGESTION WITH VARIOUS ENZYMES OR 6 *N* HCL

Amino acid*	Collagenase + imidodipeptidase	Collagenase + aminopeptidase	Collagenase + imidodipeptidase + aminopeptidase	6 *N* HCL
	Residues per 1000 residues			
HyPro	trace	0	20.0	75.6
Asp	0	0	0	47.0
Thr	0	1.75	2.75	25.6
Ser	0	2.72	4.50	26.6
Glu	0	0	0	74.5
Pro	trace	4.79	10.6	112
Gly	14.2	49.6	67.3	360
Ala	2.35	12.4	26.8	124

*Only selected amino acids are shown here; free phenylalanine and leucine also appeared in collagenase digests treated further with aminopeptidase. Additional amounts of these amino acids appeared when both aminopeptidase and imidodipeptidase were used.

quantities of free amino acids. It may be seen that a large amount of free glycine appeared (49.6 residues per 1000), a finding that was expected because *N*-terminal glycine predominates in peptides resulting from collagenase action (about 97 per cent of the total *N*-terminal residues are glycine). It is also apparent that incubation for 18 hours with collagenase and aminopeptidase caused approximately one third of the peptides to yield their *N*-terminal glycyl residues as free glycine. Of great interest is the relatively large quantity of free alanine after this treatment. Alanine could originate from further degradation of the 2 to 3 per cent of *N*-terminal alanyl peptides observable after digestion with collagenase, or from alanyl residues present in the second position of peptides having sequences of Gly-Ala-Gly- and Gly-Ala-Ala- as suggested by Grassmann and his co-workers.[10] Finally, free alanine might arise from cleavage of the dipeptide Pro-Ala, originating from a primary cleavage of the tripeptide Gly-Pro-Ala by aminopeptidase. As expected, the results also showed a large amount of free proline.

Treatment of collagenase digests of ichthyocol gelatin with both aminopeptidase and imidodipeptidase, as also shown in TABLE 1, yielded significant increases in free glycine, alanine, and proline; most dramatically it caused appearance of free hydroxyproline in large amount. As with aminopeptidase, not all aspects of specificity of imidodipeptidase are established (too few synthetic substrates have been tested), so that one cannot explain all encountered results without additional kinetic studies (appearance of individual free amino acids with varying times of digestion). A striking feature of the analysis of products of digestion of collagen by the three enzymes was the absence of free

glutamic and aspartic acids, suggesting their primary occurrence in sequences more remotely located in the collagen molecule with respect to the imino acids affected by collagenase and imidodipeptidase.

A final aspect of collagenase activity to be considered now is the nature of material that is "nondialyzable" after gelatin is treated with enzyme. Several investigators have noted the existence of such material when either collagen or gelatin is the substrate, and it has been suggested[15] that features of secondary structure are retained in peptides found after action of collagenase. Other workers,[16] using end-group analytical methods, find evidence of three-chained peptides after action of trypsin on gelatin. We shall now describe results of an experiment bearing on the composition of the "nondialyzable" fraction after collagenase action.

Gelatin derived from ichthyocol was allowed to digest with collagenase until no further increase in reactivity toward ninhydrin was observed. The digest was transferred to a cellophane sack and dialyzed at 7° C.; material remaining in the sack was sampled periodically and analyzed. The kinetic aspects of this study will be presented elsewhere, but in TABLE 2 we summarize the analysis of undialyzed material at a time (48 hours) when *change* in composition was minimal, although small amounts of material continued to leave the sack. This fraction of the digest constituted 10 per cent of the total nitrogen before initiation of dialysis. Of particular interest is the observation that the material remaining after dialysis was enriched in its contents of glutamic acid,

TABLE 2

COMPARISON OF AMINO AMINO ACID COMPOSITION OF WHOLE DIGEST OF GELATIN WITH COMPOSITION OF SAME DIGEST AFTER EXTENDED DIALYSIS

Amino Acids Determined after Hydrolysis with 6 *N* HCl for 20 Hours at 105° C.

Amino acid	Before dialysis	After dialysis
	Residues per 1000 residues	
Gly	345.9	336.0
Ala	120.6	106.5
Leu	21.2	11.9
Lys	24.6	46.6
Arg	44.4	34.8
Pro	124.8	64.0
HyPro	74.3	56.5
Asp	45.2	86.4
Glu	72.5	116.6
Tyr	2.5	10.4
Carbohydrate*	2.8	12.0

* Calculated as glucose equivalents.

aspartic acid, and lysine, and impoverished in its contents of proline and hydroxyproline when compared with the digest before dialysis. Additional striking features are the approximately fourfold increases in tyrosine and "neutral" carbohydrate contents.

Dinitrophenylation of the undialyzed material followed by analysis of the peptides by paper chromatography and paper electrophoresis ("finger-printing") revealed at least 30 distinct peptides. Only *N*-terminal glycine, as detected by the dinitrophenylation method, was encountered in these peptides: an observation that is in accord with the postulated specificity of collagenase.

The analysis suggests that the material remaining undialyzed may consist of the polar "amorphous" domains of the collagen molecule, including the tails suggested by Hodge and Schmitt.[17] The finding of large amounts of tyrosine in this fraction is in agreement with this inference. The increase in carbohydrate content of this fraction (2 per cent as compared with 0.5 per cent in the original gelatin) suggests occurrence of cross linkages, perhaps through di-ester bonds.[18,19] In any event the undialyzed portion of the collagen digest appears to contain regions full of possibilities for occurrence of one or more of the unusual linkages postulated for the collagen molecule (ester bonds, gamma glutamyl peptide bonds, and epsilon lysyl peptide linkages). At present we are exploring this fraction further by treatment with hydroxylamine, hydrazine, gamma glutamyl peptidases, and other experimental devices to define its nature.

In summary, then, we have studied the specificity of collagenase of *Clostridium histolyticum* and, with other workers, have defined at least the major sequence in collagen that is affected by the enzyme. It cannot be ruled out at this point that minor sequences of amino acids, in particular some involving alanine, are also susceptible to attack by collagenase. About 10 per cent of gelatin treated with collagenase remains undialyzed after dialysis for 48 hours, and this fraction is rich in polar amino acids and relatively poor in imino acids; it also has most of the contents of tyrosine and carbohydrate present in the original gelatin.

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Tomashefsky, Philip, M.S., Biochemistry. Biochemist & Assistant to Director, Funk Foundation for Medical Research, New York, N.Y.

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Triplett, William C., M.D., Design and Development of offshore drilling equipment, President, Sea-Mole, Inc. Corpus Christi, Texas.

Turolla, Enrico, M.D., Pathologic Anatomy. Assistant, University of Pavia, Pavia, Italy.

Vandiver, Harry Shultz, Sc.D., Mathematics. Professor, The University of Texas, Austin, Texas.

Walburg, Harry E., Jr., D.V.M., Veterinary Medicine. Fellow, United States Public Health Service, Urbana, Ill.

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Weiller, Pierre F., M.D., Pneumo-phthisiology. Clinic of Thoracic Surgery,

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Weiner, Robert Gordon, M.D., Internal Medicine. Private Practice, Halstead, Kans.

Wheelwright, Joseph Balch, M.D., Psychiatry. University of California, San Francisco, Calif.

Williams, Theodore J., Ph.D., Automatic Control. Engineering Supervisor, Monsanto Chemical Co., St. Louis, Mo.

Wiss, Oswald, M.D., Biochemistry. Director, Hoffmann La Roche & Co., Basle, Switzerland.

Wormall, Arthur, D.Sc., Biochemistry. Professor of Biochemistry, St. Bartholomew's Hospital, London, England.

Yun, Il Sun, Ph.D., Pathology. President, Seoul National University, Seoul, Korea.

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Rudolph, Michael, B.S., Medical Research. Student, Polytechnic Institute of Brooklyn, Brooklyn, N.Y.

Shabunin, Olga, Anthropology. Undergraduate Student of Anthropology, Hunter College, New York, N.Y.

NOTICE TO ACTIVE MEMBERS

In accordance with the Bylaws of the Academy, Article VIII, Section 5, the Nominating Committee of the Council is inserting in this April issue a notice to Active Members, quoting Article V, Section 1 of the Bylaws.

Article V, Section 1 reads as follows:

"**Suggestions by the Members for Nominations.** Suggestions for nominations of Officers of the Academy and Members of the Board of Trustees and of the Scientific Council may be sent in writing by any Active Member of the Academy, addressed to the Recording Secretary, with the name of the Member offering such suggestion. To be considered, such suggestions must be received not later than September 1 of each year."